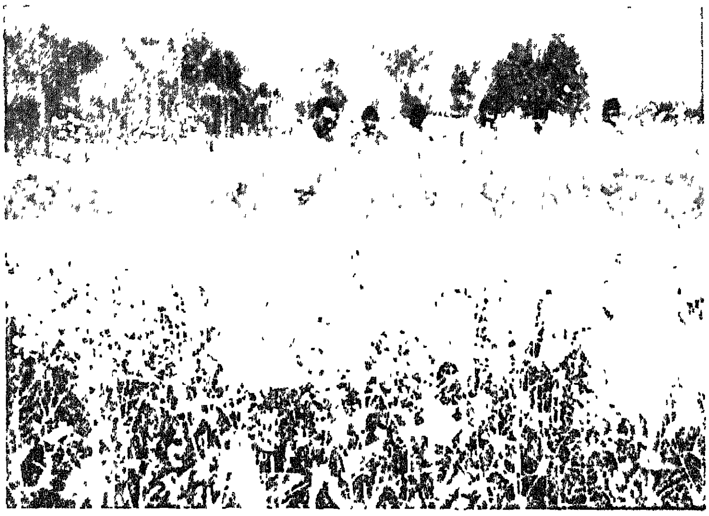
Final report of the project

Development of double low varieties of rapeseed and mustard

(December 1992 - September 2000)



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Executive Summary

Executive summary

India is the third largest oilseed producing country in the world. Brassicas (rapeseed-mustard) are the second most important edible oilseed crop in India, next only to groundnut and constitute important species grown for vegetables, condiments and edible oil. The oil is mainly used for edible purposes accounting for nearly 30% of the total oilseeds produced in the country. The fatty acid composition of the oil determines its nutritional quality. The rapeseed oil contains lowest amount of saturated fatty acids as compared to other vegetable oils. It contains nutritionally desired oleic acid, which gives stability to the oil, along with two essential fatty acids, linoleic and linolenic. However, in addition, it also contains high amounts of erucic acid, which is nutritionally undesired. The deoiled cake is a rich source of proteins and minerals. It has well balanced amino acids and vit. E. It is mainly used as animal feed. However, it contains high amounts of sulphur containing compounds (glucosinolates) which along with its degradation products have adverse effects on animals' productivity and is thus considered harmful for consumption for feed purposes (Kumar and Tsunoda, 1980).

Considering the adverse effects of diet rich in erucic acid and glucosinolates (Gopalan et al. 1974, Bille et al. 1983), efforts were initiated at global level towards improving nutritional quality of rapeseed-mustard oil and cake. The international efforts for developing rapeseed mustard strains with single low/ double low characteristics were initiated during early fiftees. Stefansson et al developed the first zero erucic acid strains of *B. napus* in 1961. This was followed by the development of zero erucic acid strains of *B. campestris* (Downey 1964). In similar studies Kirk and Oram (1981) obtained low erucic acid strains of *B. juncea* by screening for low erucic acid in the F_2 progeny by half seed technique. Alonso et al. (1991) have obtained the low erucic acid genotypes of Ethiopian mustard through continuous pedigree selection of plants with reduced erucic acid.

For the first time in 1967 Polish *B. napus* cultivar `Bronowski' was discovered containing glucosinolate content of about 12 μm/g oil free meal. It also contained low erucic acid in the seed oil (7-10%). So far the `Bronowski' gene is the only known source for low glucosinolate content which has been utilized for the development of canola quality cultivars. `Canola' (commonly known as `double low' or `00') is a trade name designating rapeseed having less than 2% erucic acid in the seed oil and less than 30 μm glucosinolate/g of the deoiled cake (Downey 1990).

The efforts towards development of double low cultivars led to the release of world's first double low *B. napus* cv. Tower by Stefansson in 1974 and the world's

first double low *B. campestris* cv. Candle by Downey in 1977 (Bell 1982). *B. napus* varieties having canola quality and high oil content such as Cyclone (from Denmark), Shiralee (from Australia) and AC Excel (from Canada) are available. During recent years, the low erucic acid/low glucosinolate/double low strains of many brassica species and its wide allies have also been reported from several countries (Scarth 1995, Rakow 1995, Potts et al. 1999, Oram et al. 1999). In addition to the low erucic acid and low glucosinolate, yellow seed coat colour is another desired characteristic, and during recent years, the concept of 'OO' is being expanded to 'OOO' to include this as one of the major breeding objectives.

In India, the Technology Mission on Oilseeds was launched during mid eightees, and as a result the rapeseed-mustard production became more than double within a decade, increasing from about 3 mT in 1985 to more than 6 mT in 1995. Until late eighties, the emphasis has mainly been on increase in the yield rather than on the quality. However, having achieved substantial increase in the quantity of rapeseed-mustard production, in recent years the emphasis is being laid for the improvement in oil and meal quality. The high amounts of erucic acid in the seed oil (40-50%) and high glucosinolate (80-160µm/g) in the oil free meal restrict the global utilization of Indian mustard oil and cake. Efforts to introduce exotic canola quality rapeseed varieties did not succeed due to non-adaptability of these exotic canola varieties to Indian growing conditions resulting in late maturity and poor seed set (Anonymous 1994). Hence, an imperative need was felt to transfer the genes for low erucic acid and low glucosinolate in the background of Indian rapeseed and mustard.

The breeding for low erucic acid and low glucosinolate becomes difficult due to the non-availability of desirable donor sources as well as the complicated genetic factors. The erucic acid content is reported to be under the control of two genes with additive effects (Harvey and Downey 1964, Kondra and Stefansson 1965, Siebel and Pauls 1989). Each seed from the same plant contains different genetic background resulting in seed to seed variation in the levels of erucic acid. Glucosinolates are controlled by maternal genotype and are governed by at least three partially recessive genes (Kondra and Stefansson 1970, Uzunova et al. 1995). Since both erucic acid and glucosinolates are inherited independently, a large number of segregating plant populations need to be screened, and the lack of precise and efficient screening techniques for erucic acid and glucosinolate may become the bottleneck for quality breeding programmes. Therefore, efficient analytical methods are a pre-requisite for selection of desired genotypes from among a large number of segregating populations (Agnihotri 1999).

The biggest challenge is to combine the double low characteristics with good yielding capability, all of which are quantitative traits governed by multiple recessive genes. The genetic engineering method to transfer the genes for low erucic acid and low glucosinolate in the high yielding varieties would have been helpful in achieving this target. However, while the work on these lines for gene identification, cloning and transformation, for reduction in erucic acid and glucosinolate, was still in nascent stage during early nineties, the work was initiated under this project to combine half seed technique and efficient analytical methods to expedite the conventional breeding methods for the purpose. The efforts have also been in progress at various ICAR centres and SAU's since about two decades. While considerably good progress has been made towards generation of low erucic acid / low glucosinolate *B. juncea* and *B. napus* strains (Khalatkar et al 1991; Malode et al. 1995; Agnihotri et al. 1995; Banga et al. 1998; Agnihotri and Kaushik 1998, 1999), the desired success is yet to be achieved and these strains need to be improved for their agronomic attributes.

Keeping in view the imperative need to transfer double low characteristics in Indian *B. juncea* and *B. napus*, the work was initiated under this project in December 1992. The improved methods of GC and HPLC were developed for efficient analysis of a large number of segregating population. The NIR has also been standardized for oil/moisture content and glucosinolate measurements. The use of off-season nursery facilities at FRL, Leh proved very helpful in generating valuable germplasm by taking two crops in a year. Many low erucic acid/low glucosinolate strains of *B. juncea* have been developed. The success has been notably impressive in *B. napus*. The low erucic acid/double low, early maturing strains of *B. napus* have been developed. The improved quality strains of *B. juncea* and *B. napus* have been contributed for multilocation testing under AICRP R&M, and also registered at NBPGR-ICAR.

The salient achievements/outcome, future scope and experimental details of the work under the NOVODB sponsored project (Dec. 1992 - Sept. 2000) are presented in the following sections.

Salient Achievements and Outcome

Figures

Publications from the Project

1. Establishment of half seed technique and improved method of GLC for fatty acids analysis (Fig. 1a, b)

It is important to use an efficient method to screen the segregating populations for their fatty acids composition. Gas chromatography is the most valuable and efficient method for analysis of fatty acid methyl esters. Sample preparation for the analysis includes oil extraction and esterification, requiring oil extraction prior to esterification. This can be eliminated by a one-step trans-esterification using acetylchloride. Further modification was achieved by replacing the conventional heating time 1 hr, using the microwave heating, and a novel packed column for separation of fatty acid methyl esters (FAMEs). An efficient and economical GC method for rapid determination of FAMEs in rapeseed-mustard has been developed. The seeds were transmethylated with acetylchloride, using microwave heating and separation achieved on a 3 m column packed with a mixture of 2% SP-2300 and 3% SP-2310 on Chromosorb `W'. The method was compared with the conventional heating method and extended efficiently for half-seed analysis. The fatty acid composition of the FAMEs mixtures prepared by both methods were similar with highly significant correlation coefficients ($P<0.001$). The advantages of the improved method for FAMEs analysis are as follows:

- Rapid Transmethylation
    - Elimination of oil extraction
    - Reduced reaction time
    - Less oxidation of PUFAs
    - No antioxidant required
    - Elimination of artifacts in chromatogram
    - Faster separation
    - Increased gas chromatographic capacity

- 2% SP-2300 + 3% SP-2310 6 ft. column
    - Faster separation
    - Increased gas chromatographic capacity

Reference:

Kaushik N, Agnihotri A. 1997

Evaluation of Improved method for determination of rapeseed-mustard FAMES by GC.

Chromatographia **44**: 97-99.

Fig.1a:

Schematic presentation of half seed technique

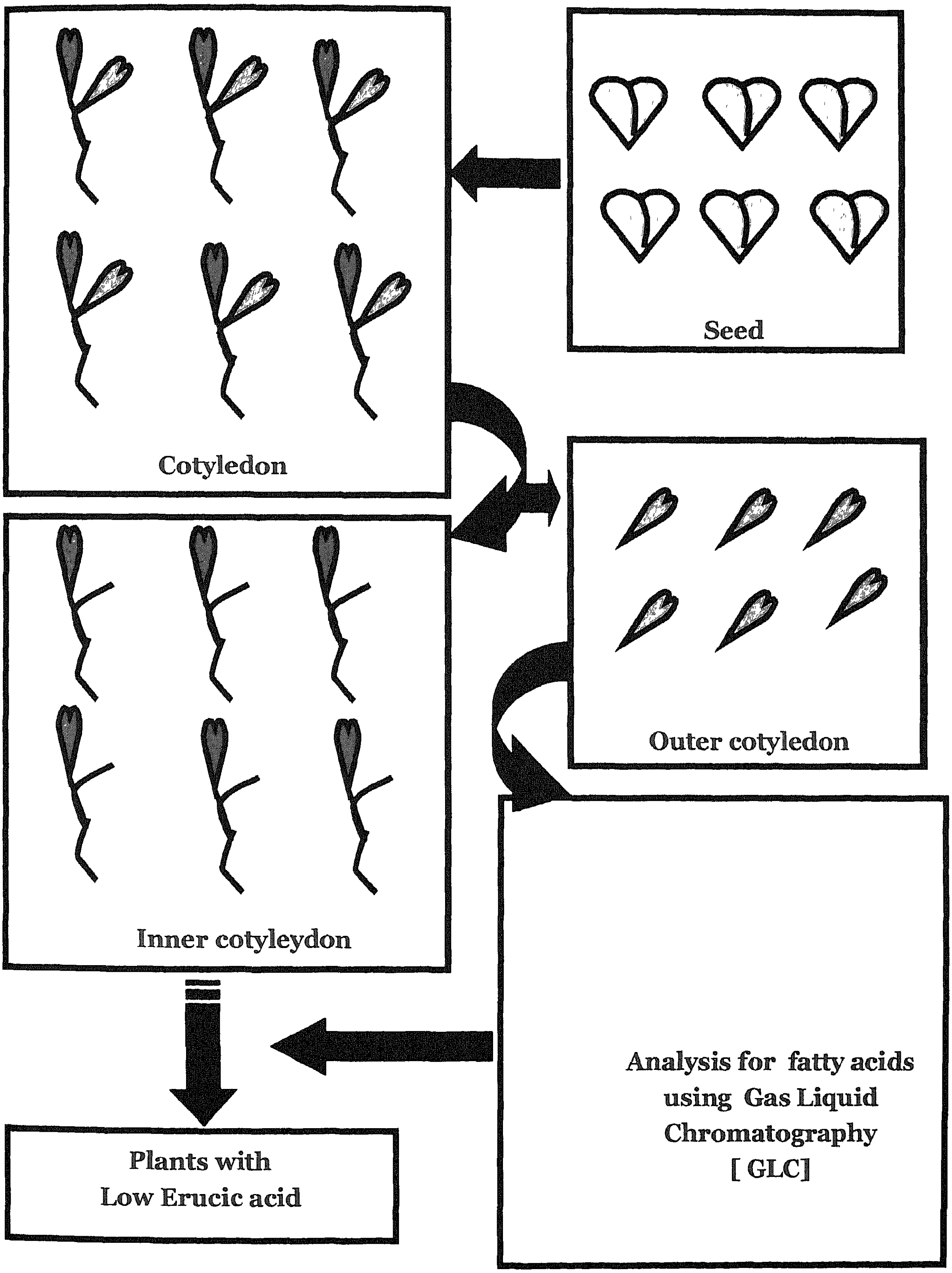
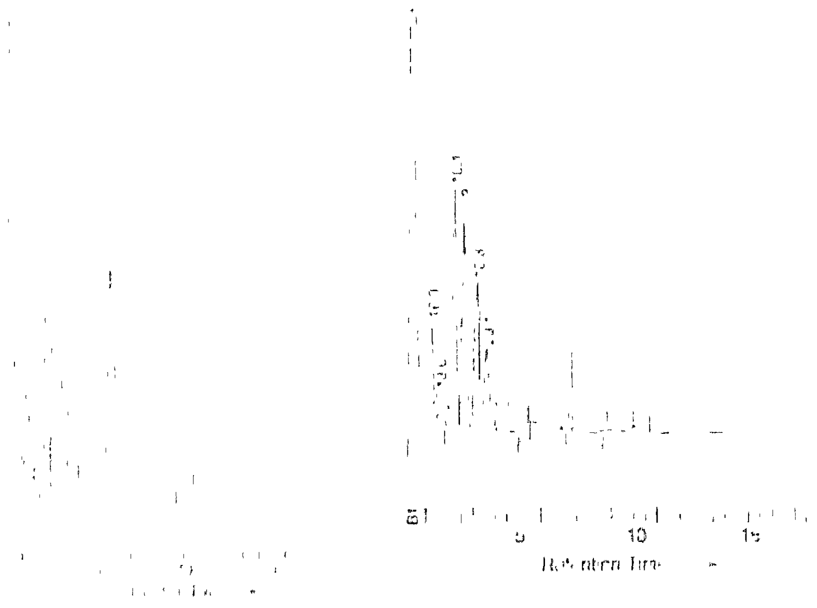

Seed
Cotyledon
Outer cotyledon
Inner cotyleydon
Analysis for fatty acids using Gas Liquid Chromatography [GLC]
Plants with Low Erucic acid

Fig. 1b:

GLC chromatogram of high and zero erucic *B. juncea*



2. Establishment of improved method of HPLC for glucosinolate analysis (Fig. 2)

Selection of plants with desired glucosinolate level largely depends on rapid and efficient screening of highly segregating plant populations. Various methods reported for the estimation of glucosinolates include test-tape, enzyme immobilization, gravimetric, spectroscopic, X-ray fluorescence, gas liquid chromatographic and high performance liquid chromatographic (HPLC) methods. Out of these HPLC has many advantages over the others. Among various existing HPLC methods, each has its own advantages or disadvantages, and separation of intact glucosinolates using specific salts in aqueous mobile phase can eliminate many of the disadvantages associated with other methods. A simple, economical and efficient HPLC method has been developed for the separation and determination of individual glucosinolates in rapeseed-mustard. The method involves single step extraction of glucosinolates in boiling water. Separation of individual glucosinolates was achieved on Novapack RP-18 column (3.9 x 150 mm), using 0.2 M ammonium sulphate as mobile phase and peaks were monitored at 229 nm. All major glucosinolates could be eluted within 10 min run time. The method can be effectively utilized for routine glucosinolates analysis. The advantages of the improved method for HPLC analysis are as follows:

- Single step extraction
- Clean up is eliminated
- Desulphatation is not required
- Rapid sample preparation
- Reduced run time
- Increased chromatographic capacity

Reference:

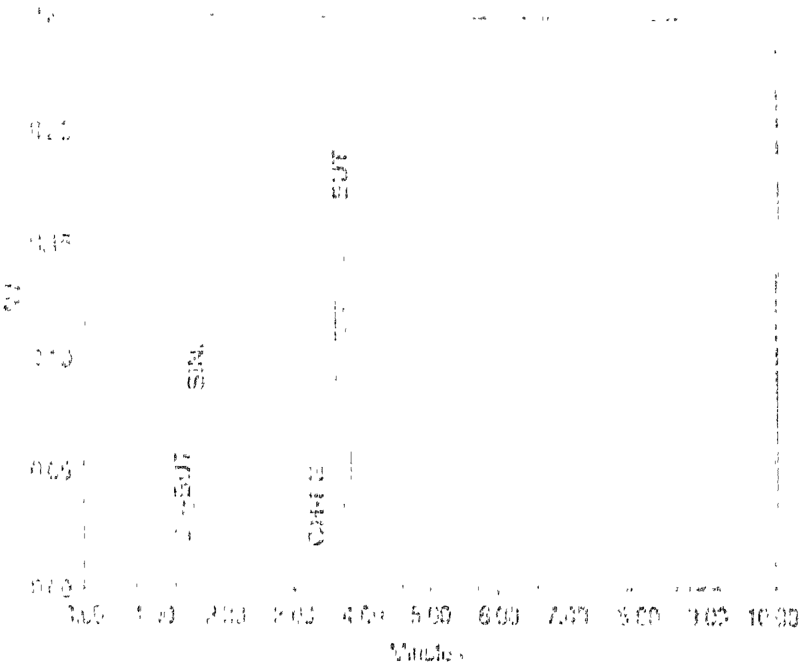
Kaushik N, Agnihotri A. 1999

High Performance Liquid Chromatographic method for Separation and Quantification of Intact Glucosinolates.

Chromatographia 49(5/6): 281-284.

Fig.2:

Overlay HPLC chromatogram of high and low glucosinolate *B.juncea*



3. Development of early maturing, low erucic acid, yellow seeded strains of *B. juncea* and early maturing, low erucic acid, dwarf strains of *B. napus* (Fig. 3)

The nutritionally improved zero erucic rapeseed mustard strains were developed from the transgressive segregants of intergeneric crosses of *Brassica* (Agnihotri et al. 1995). Single plant selections were made for four consecutive generations for phenotypically superior early maturing plants following the pedigree method. The plants were analyzed for their fatty acids through bulk seed analysis, followed by half seed technique. They have zero erucic acid and high oleic acid, 40-50% in *B. juncea* and 45-70% in *B. napus*, as compared to the high erucic acid (40-50%) and low oleic acid (10-25%) in the cultivated varieties. The essential fatty acid, linoleic acid, is much higher, 30-38% in *B. juncea* and upto 22% in *B. napus*, as compared to 10-15% in the national check varieties Varuna and GSL-1. The oil content of some of these lines viz. TERI(OE)R05, R09, M08 and M10, is marginally higher than the national check varieties. A significant reduction in the days to maturity was observed in a large number of segregants. Some of the selected zero erucic acid strains of *B. napus* viz TERI(OE)R03 and TERI(OE)R15 and *B. juncea* viz. TERI(OE)M09 and TERI(OE)M21, matured 17 to 35 days earlier than the national check varieties. Several of these low erucic acid strains were tested in IVT-1 under AICRP R&M. Three zero erucic acid strains of *B. napus* are in AVT-1. The early maturing, low erucic acid *B. napus* strain TERI(OE)R03 has been promoted to AVT-II.

Reference:
Agnihotri A, Kaushik N. 1998
Transgressive segregation and selection of zero erucic acid strains from intergeneric crosses of Brassica
Ind. J. of Plant Genetic Resources **11**(2), 251-255.

Transgressive segregation and selection of zero erucic acid strains of *B. juncea* and *B. napus*

- (*E. sativa* x *B. campestris*) x *B. juncea*
- (*B. napus* x *Raphanobrassica*) x *B. napus*
  - *In vitro* embryo rescue
  - Callusing / embryogenesis
  - Plantlet regeneration
  - Selection for low erucic acid plants through half seed analysis
  - Recurrent selection - SPP
  - Progeny advancement - pedigree method

Low erucic acid, early maturing,
dwarf *B. napus* (TERI-OE-R05) on left and
B. napus var. GSL-1 (NC) on right

4. Transfer of low erucic acid content in *B. juncea* var. Varuna and Pusa Bold (Fig. 4)

The popularly grown high yielding *B. juncea* var. Varuna and Pusa Bold were chosen as a female parent for transfer of low erucic acid content from the zero erucic acid donors T-7 and Zem-1. The F_2 seeds were subjected to half seed analysis. The single plant progenies, derived from the remaining half cotyledon of the identified zero erucic acid seeds, selected for better proportion of oleic, linoleic and linolenic acids, were utilized for development of zero erucic acid strains following the pedigree method. Agronomical selections were made during four subsequent generations. The zero erucic acid strains of *B. juncea* var. Varuna viz. TERI(OE)M981 and TERI(OE)M982, and of *B. juncea* var. Pusa Bold TERI(OE)M9903 were contributed to AICRP R&M for multilocation testing. The data indicates that there is a need to improve the agronomic characteristics for better yielding capability.

Reference:

Agnihotri A, Kaushik N. 1999

Incorporation of superior nutritional quality traits in Indian *B. juncea*

Indian Journal of Plant Genetic Resources **12**(3) (In press)

Fig. 4.

Low erucic acid *B. juncea* var. Pusa Bold

Low erucic acid *B. juncea* var. Varuna

5. Transfer of low glucosinolate and double low characteristics in Indian *B. juncea* var. Varuna (Fig. 5)

The low glucosinolate exotic donor of *B. juncea* BJ-1058 and low erucic acid strain Zem-1 was used to transfer the low glucosinolate and double low characteristics in *B. juncea* var. Varuna. A unique three way cross *B. juncea* (var. Varuna x Zem-1) x BJ-1058 was made for the transfer of 'OO' characteristics. The F_3 seeds were analyzed for their glucosinolate content through HPLC and several plants were identified having 25-30 μm glucosinolate/g oil free meal. The seeds of low glucosinolate plants from the three way cross were subjected to half seed technique and analyzed through GC for low erucic acid. The progenies of the selected plants were advanced through pedigree method and recurrent selections. In the single plant progenies of low glucosinolate selections, some of the plants again segregated to synthesize more than 30 μm glucosinolate during $F_5/F_6/F_7$ generations. Selections were made where most of the plants synthesized <30 μm glucosinolate and the plants having low (18-22 μm) glucosinolate contents were used for progeny advancement. Poor recovery of low glucosinolate plants is due to the involvement of several recessive genes resulting in segregation for high glucosinolate types even upto F_6/F_7 generation. The agronomic characteristics were recorded. The low glucosinolate plants are early in maturity (132-140 days) having shy branching as compared to the double low plants (142-152 days) with profound vegetative growth. Thus, there is a need for agronomic improvement of these lines through bi-parental mating and selection for desired agronomic attributes along with low erucic acid and low glucosinolate characteristics. Since no *B. juncea* germplasm is available in India having low glucosinolate/double low characteristics, these strains will serve as valuable genepool for evolving nutritionally superior *B. juncea* with good agronomic attributes.

Reference:
Agnihotri A, Kaushik N. 1999.
Genetic enhancement for double low characteristics in Indian rapeseed mustard
Proceedings, Xth International Rapeseed Congress, September 26-29, 1999, Canberra, Australia.

Fig. 3:

Transfer of low glucosinolate and double low characteristics in Indian *B. juncea*

Low Glucosinolate

B.juncea var. Varuna
(High Erucic Acid
High Glucosinolate)

B.juncea line BJ-1058
(Low glucosinolate,
High erucic acid)

F_1 seeds

HPLC analysis of F_3 seeds
for selection of low glucosinolate

Selection for low glucosinolate

Double Low

B.juncea var. Varuna
(High erucic acid
High glucosinolate)

B.juncea var. ZEM-1
(Low erucic acid,
High glucosinolate)

F_1 seeds x *B. juncea* line BJ-1058

F_3 seeds
Selection of low glucosinolate (HPLC)
followed by half seed analysis for
low erucic acid selection (GLC)

Double low *B. juncea* var. Varuna

6. Development of double low varieties of *B. napus* (Fig. 6)

The early maturing *B. napus* lines TBN-1 and TBN-5, derived from advanced generation backcross progeny of (*B. napus* x *Raphanobrassica*) x *B. napus* (Agnihotri et. al. 1990) and selected for zero erucic acid through half seed technique (Agnihotri et. al. 1995) were used as female parent. The exotic double low *B. napus* var. Shiralee, Regent and Cyclone were used as pollen donors for the transfer of double low characteristics. The plants were grown to maturity, the F_1 and F_2 seeds were harvested. The plants were bagged, and selfed and open pollinated (OP) F_3 seeds were collected from a large number of individual plants. The F_3 OP seeds were analyzed for their glucosinolate content by the improved HPLC method. The selfed seeds of the plants, showing less than 30μm glucosinolate/g oil free meal, were utilized to grow single plant progenies. During the next three subsequent generations the single plant progenies were grown, and selfed and OP seeds were harvested from the individual plants. The glucosinolate content was analyzed in each generation to select plants having glucosinolate less than 30μm/g oil free meal. Simultaneously the plants were selected for early maturity in each generation.

The F_6 selfed seeds of the plants having less than 30μm glucosinolate/g oil free meal, were analyzed for their fatty acids content determined by the improved GLC method. The plants having less than 2% erucic acid in the seed oil and less than 30μm aliphatic glucosinolate/g oil free meal were identified for further progeny advancement. In general, the plants derived from TBN-1 x Regent were early in maturity (120 to 130 days) as compared to those derived from crosses TBN-5 x Cyclone (125 to 145 days).

The newly developed double low strains of *B. napus* having zero erucic acid in the seed oil and low glucosinolate (12-15 μm/g) in the oil free meal have compact plant type and shorter maturity period (125-140 days) as compared to the widely grown national check *B. napus* var. GSL-1 having maturity period of 153 days. Three of these strains were tested under AICRP R&M for their yield evaluation. They were either at par or performed better than the *B. napus* national check var. GSL-1 continuously for two years in IVT-1 and have been promoted to AVT-1.

Reference:

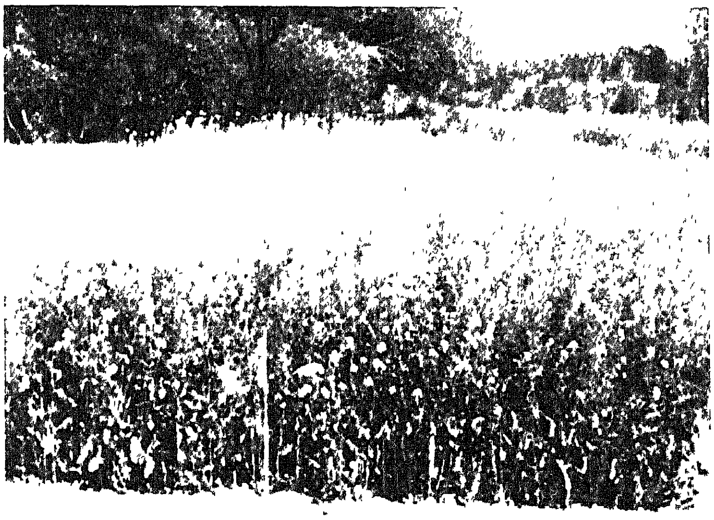
Agnihotri A, Kaushik N. 1999

Transfer of double low characteristics in Indian *B. napus*

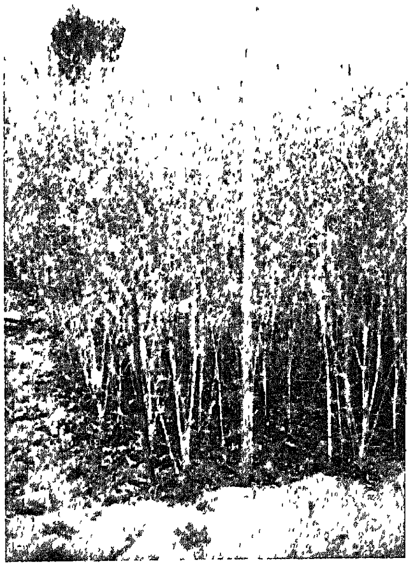
Journal of Oilseeds Research **16**(2): 227-229.

Fig. 10a

Double low *B. napus* at TERI field station, Gual Pahari, Gurgaon



TERI- Gaurav (INGR 99007)



TERI- Garima (INGR 99008)

7. The status of low erucic acid/low glucosinolate/double low strains developed at TERI.

Lines	Range of erucic acid (%)	Range of glucosinolate µm/g deoiled cake	Days to Maturity	Status
B. juncea				
Varuna x T-7/ZEM	0.0 - 1.5	--	134-136	low erucic acid
Pusa Bold x T-7/ZEM	0.0 - 1.2	--	134-136	low erucic acid
T-7 x BJ-1058	0.0 - 46.0	16-21	130-136	low erucic / low glucosinolate
Varuna x BJ-1058	48.0 - 50.0	18-22	132-135	low glucosinolate
(Varuna x Zem-1) x BJ-1058	0.0 - 44.0	15-22	145-153	double low
B napus				
T-18 x Shiralee	0.0 - 1.5	10-17	130-135	double low
T-19 x Regent	0.0 - 1.0	10-23	125-132	double low
T-18 x Cyclone	0.0 - 1.8	8-16	130-143	double low

Several of these strains are being evaluated under AICRP R&M. Seven low erucic acid/double low strains of *B. napus* are under AVT-I/AVT-II.

8. Enhanced quality strains registered at NBPGR

- SWARNA-TERI(OE) M21-INGR 98001; Zero erucic acid, yellow seeded, early maturing (117 days), *B. juncea*
- PHAGUNI-TERI(OE) R03-INGR 98002; Zero erucic acid, early maturing (136 days), *B. napus*
- SHYAMALI-TERI(OE) R09-INGR 98005; Zero erucic acid, high oleic (70.1%), *B. napus*
- GAURAV-TERI(OO) R986-INGR 99007; Zero erucic acid, low glucosinolate (15.3 μm/g oil free meal), early maturing (125 days), dwarf double low *B. napus*
- GARIMA-TERI(OO) R985-INGR 99008; Zero erucic acid, low glucosinolate (12.2 μm/g oil free meal), high oleic acid (57%), double low *B. napus*

The improved quality rapeseed-mustard strains developed at TERI and registered at NBPGR, ICAR are the first quality lines to be registered in India.

Reference:

Anonymous 1999
Plant germplasm registration notification
Indian J. Genet. **59**(3): 387-391.

Anonymous 1998
Plant germplasm registration notification
Indian Journal of Plant Genetic Resources **11**(1): 121-122

List of publications from the project

No. of Publications *12*

Agnihotri A, Kaushik N. 1999
Incorporation of superior nutritional quality traits in Indian *B. juncea*
Indian Journal of Plant Genetic Resources, **12**(3) (In press)

Agnihotri A, Kaushik N. 1999
Transfer of double low characteristics in Indian *B. napus*
Journal of Oilseeds Research **16**(2): 227-229.

Agnihotri A, Kaushik N. 1999
Genetic enhancement for double low characteristics in Indian rapeseed mustard
Proceedings, Xth International Rapeseed Congress, September 26-29, 1999, Canberra, Australia.

Kaushik N, Agnihotri A. 1999
High Performance Liquid Chromatographic method for Separation and Quantification of Intact Glucosinolates
Chromatographia **49**(5/6): 281-284.

Agnihotri A, Kaushik N. 1998
Transgressive segregation and selection of zero erucic acid strains from intergeneric crosses of Brassica
Ind. J. of Plant Genetic Resources **11**(2), 251-255.

Kaushik N, Agnihotri A. 1997
Evaluation of Improved method for determination of rapeseed-mustard FAMES by GC
Chromatographia **44**: 97-99.

Agnihotri A, Kaushik N. 1996
Transfer of double low characteristics in early maturing B. napus lines
Cruciferae Newsletter **18**: 74-75.

Agnihotri A, Kaushik N. 1996
Transfer of double low characteristics in Indian *B. juncea*
Cruciferae Newsletter **18**: 86-87.

Singh N K, Kaushik N, Sarkar G, Agnihotri A. 1996
Variation and association analysis among major fatty acids in rapeseed-mustard
Cruciferae Newsletter **16**: 88-89.

Kaushik N, Agnihotri A. 1996
Improved analytical methods: An aid for selection of rapeseed - mustard having better nutritional quality
Abstract 2nd International crop Science Congress, New Delhi, India. November 17-24, 1996.

Kaushik N, Sarkar G, Singh N K, Agnihotri A. 1995
An elegant method for rapid separation of fatty acid esters by packed column gas liquid chromatography
Proceedings, 18th International Conference on Science and Technology, New Delhi, India. November 16-17, 1995.

Kaushik N, Singh N K, Agnihotri A. 1995
Separation of intact glucosinolates by HPLC and study on the characteristics of glucosinolates in Indian mustard Murphy, D. (ed).
Rapessed Today and Tomorrow **3**: 916-918.

Future Scope

Future scope

(a) In India, the area under *B. napus* cultivation has been restricted mainly due to late maturity and has great potential for expansion. The newly developed nutritionally improved quality (low erucic acid/double low), early maturing, dwarf *B. napus* strains offer great potential for *B. napus* expansion in non-traditional areas of India. Several of these strains are in advanced stages of testing (AVT-I / AVT-II) under AICRP R&M of ICAR.

(b) In India no *B. juncea* germplasm having double low (low erucic acid and low glucosinolate) characteristic is available as of today. Hence the low glucosinolate / double low *B. juncea* strains developed under this project, will serve as a valuable germplasm for use in the breeding programme as *B. juncea* donor for development of double low *B. juncea* suitable for Indian growing conditions.

(c) The low erucic acid oil is important for human nutrition and there is a need to generate public awareness and promote low erucic acid oil for the benefit of the society. The low glucosinolate meal is important for animal nutrition and has a high demand in the international market. Presently, India is exporting rapeseed-mustard meal at a much lower rate, and can easily attract substantial increase in the revenue in international market by exporting meal from the low glucosinolate strains having internationally acceptable levels of less than 30 µm/g deoiled cake. However, cultivation of quality lines in the rapeseed-mustard growing areas may lead to cross contamination in the fields. The maintenance of purity and monitoring of quality status is of utmost importance for replacement of existing high erucic acid, high

glucosinolate containing rapeseed-mustard cultivars in a phased manner. Also, there is a need to evolve a strategy to develop linkages with the oil companies/millers for marketing, and a buy back arrangement of quality seeds from the farmers.

Project Background & Financial Support

Co-ordination and Progress Reviews

Project Background

Rapeseed mustard is the second most important edible oilseed crop in India. The Indian cultivated varieties of rapeseed and mustard contain high erucic acid (40-50%) in the seed oil and high glucosinolate (80-160 µm/g) in the oil free meal against the internationally accepted and recommended levels of <2% erucic acid in the seed oil and <30 µm glucosinolate/g in the oil free meal (Canola quality). Efforts to introduce exotic canola quality cultivars in India did not succeed due to their non-adaptability to Indian agro-climatic conditions (Anonymous 1994). Keeping in view the undesired effects of erucic acid and glucosinolate for human consumption and animal feed respectively (Gopalan *et al.* 1974, Bille *et al.* 1983), and in order to meet the international standards, transfer of double low characteristics in Indian rapeseed-mustard was identified as a priority area by TMOP.

At TERI the work on brassica biotechnology, initiated during late eightees, involved wide hybridization in Brassicas for incorporation of useful agronomical characteristics from the wide allies into the cultivated varieties. *B. juncea* lines derived from (*E. sativa* x *B. campestris*) amphidiploid x *B. juncea*, were selected for better plant type and yield (Agnihotri et. al. 1990a). *B. napus* lines derived from (*B. napus* x *Raphanobrassica*) x *B. napus* were selected for early maturity and shattering resistance (Agnihotri et. al. 1990b). Preliminary analysis of *B. juncea* and *B. napus* lines, obtained through *in vitro* embryo rescue technique (Agnihotri 1993a), and derived from the advanced generation backcross progenies, indicated the possibility of getting low erucic acid or low glucosinolate in some of these lines (Agnihotri 1993b). With this background, the NOVOD Board was approached with the present proposal for transfer of double low characteristics in Indian rapeseed mustard and the project was sanctioned w.e.f. December 1992. The progress made so far is discussed herewith.

Financial support

The sanctioned budget for the period Dec. 1992 - Nov. 1997 and for the extension period i.e. Dec. 1997 to March 2000 is given in Annexure-1. The reappropriated budget for the period 1998-99 and 1999-2000, and the sanctioned budget for the extension period Apr. to Sept. 2000 is given in Annexure-2. The component wise yearly expenditure for the period Dec. 1992 to Sept. 2000 is given in Annexure-3. The list of equipments purchased under the project is indicated in Annexure-4. The approved technical programme is enclosed as Annexure-5.

Co-ordination and Progress Reviews

The progress of the project was reviewed regularly by an expert group comprising of experts from Ministry of Agriculture, ICAR and SAUs. Two national reviews of all the centres working on development of double low varieties of rapeseed and mustard were also held. The first joint review of progress on the development of `0-0' varieties of rapeseed and mustard was held under the chairmanship of Dr. R. S. Paroda, DG, ICAR, on 20.2.95 at NOVODB. The meeting was attended by DDG(CS) and ADG(OP), ICAR; E.D. and Secretary NOVODB; Director, NRC R&M; industry representatives including President and Secretary SEA; Scientists from TERI, NDUA&T, GBPUA&T, PAU and Nagpur University.

The second joint review meeting of the scientists working on `00' rapeseed-mustard was held at NDDB, Anand on December 2-3, 1996 under the co-chairmanship of Dr. E. A. Siddiq, DDG(CS), ICAR and Dr. (Ms.) Amrita Patel, MD, NDDB to review the status of work being carried out at different centres and to plan future strategy.

In addition, the work progress under the aforesaid project was also reviewed on 10.7.97 under the Chairmanship of Shri K. Rajan, Secretary (A &C) and Chairman, Managing Committee, NOVODB, at Krishi Bhawan, New Delhi for consideration of appropriate extension of the project. As desired, Dr. Abha Agnihotri, PI, made a presentation giving background of the project, status of progress since implementation of the project and the agronomical and quality characteristics of the material developed. The expert members of the review committee, Shri J.N.L. Srivastava, Addl. Secreatry and Director TMOP; Shri K. Arya, Joint Secretary TMOP & ED, NOVODB; Dr. N.B. Singh, ADG (OP), ICAR; Dr. J.P. Singh, Secretary, NOVODB participated in discussions and expressed their views. The Chairman stated the need for early development and promotion of Canola quality cultivars. He also emphasized the priority for the development of quality material to be further utilized by breeders/institutions for development of high yielding `00' varieties. In view of the commendable progress made so far, the Chairman favoured for extension of project upto March, 2000.

The sixth and seventh reviews of progress of NOVODB sponsored project were held during Feb. 98 and March 1999 respectively, under the chairmanship of Dr. Mangla Rai, DDG(CS), ICAR. The expert members of the review committee, Dr. Basudeo Singh, Executive Director, NOVODB, Dr. N.B. Singh, ADG(OP), ICAR, and Dr. P.R. Kumar, Director, NRC R&M complimented TERI for the development of zero erucic acid lines of *B. juncea* and *B. napus* and the efforts towards development of low glucosinolate and double low genotypes. The expert group

appreciated the progress made by TERI under this project so far. The progress of the work till December 1999 was reviewed by an expert committee chaired by Dr. Mangla Rai, DDG(CS), ICAR on December 30, 1999. The expert group recognized the good work accomplished under the project by development of potential donors for subsequent breeding programmes. Accordingly, in order to bring the project to a logical conclusion, the project was extended upto Sept. 2000 with an additional outlay of Rs. 3.83 lakhs for harvesting, recording of agronomical data, quality analysis and compilation of final report.

The progress under the NOVODB sponsored project on "Development of double low varieties of rapeseed and mustard" for the period December 1992 - till September 2000 is herewith discussed. The year wise experimental details, recommendations of the review meetings and action taken are presented in the following sections.

The work progress under the project being reviewed by expert members of ICAR and Ministry of Agriculture, at TERI field station, Gual Pahari, Gurgaon.

Rabi 1996-97

Rabi 1997-98

Year-wise Experimental Details

Tables

Year wise experimental details

December 1992 - March 1993

A large number *B. juncea* plants derived from advanced generation backcross progeny of (*E. sativa* x *B. campestris*) amphidiploid x *B. juncea* and *B. napus* plants derived from advanced generation backcross progeny of (*B. napus* x *Raphanobrassica*) x *B. napus* along with indigenous and exotic lines/cultivars of *B. juncea* and *B. napus* were grown at TERI field station, Gual Pahari, Gurgaon, following the recommended agricultural practices. Individual plants of each line/cultivar were selected and bagged for selfing. The self and OP seeds were harvested separately.

April 1993 - March 1994

(a) The initial glucosinolate analysis was performed by the tes-tape method (Table 1,2). None of the Indian cultivars was found to have low glucosinolate.

(b) The fatty acid composition of the seed oil was analysed by gas chromatography (Table 3). None of the Indian cultivar was found to have low amount of erucic acid in the seed oil. However, some of the selected *B. juncea* and *B. napus* lines identified at TERI had low levels of erucic acid (Table 4,5).

(c) The plants showing less than 20% of erucic acid were analysed by half seed method. Some of the plants were selected for less than 2% erucic acid and were grown further for the multiplication of seeds.

(d) A large number of crosses were made using the Indian cultivated varieties and exotic varieties of *B. juncea* and *B. napus* for transfer of low erucic and low glucosinolate characteristics in Indian cultivars. The *B. juncea* and *B. napus* lines identified at TERI were also used for crossing with exotic 0/00 germplasm for the purpose and a large number of crosses were attempted (Table 6).

(e) A novel rapid method for analysis of fatty acids by GLC was developed and standardized.

(f) The seeds of F_1s and reciprocal crosses were collected separately for both *B. juncea* and *B. napus* crosses.

April 1994 - March 1995

(a) The F_1 seeds of various crosses were analysed for the fatty acid composition (Table 7). The *B. juncea* plants were intermediate type, ranging from 24% to 35% for erucic acid content. The erucic acid content of F_1 seeds of *B. napus* ranged from 0 to 35%.

(b) The F_1 seeds of some of the crosses alongwith their parents of both *B. juncea* and *B. napus* were grown at Leh during April-September 1994 for advancement of generation. Several backcrosses and selfing of F_1 plants of each cross were performed. The selfed F_2 seeds and backcross seeds were harvested and collected separately.

(c) The F_2 seeds of Pusa Bold x ZEM and Pusa Bold x T-7 were analyzed by half seed technique. The erucic acid content in the cross of Zem-1 ranged from 1 to 55% and in that of T-7 ranged from 0 to 50%. The seeds having less than 15% erucic acid were selected. The selected plants were grown from the remaining half seed.

(d) The remaining F_1s, F_2s and backcross seeds of different crosses including their parents were grown at Gual Pahari during 1994-95. The plants were selfed and crossed for undertaking further work as per the technical programme.

(e) A suitable method was developed for separation of glucosinolates by HPLC.

April 1995 - March 1996

(a) The method of fatty acid analysis by GLC was further improved for efficient analysis of seeds. The method of glucosinolate analysis by HPLC was also standardized to satisfaction for rapid quantitative separation of glucosinolates. NIR was procured and installed. The process of standardization of NIR for glucosinolate and oil/moisture content was initiated.

(b) The anthers from some of the F_1 plants were collected and cultured *in vitro*. Some of the anthers showed callusing, however, none of the cultures could be induced to develop embryos or regenerate plantlets on various combinations of media/growth supplements tested. This could be due to the lack of proper and uniform physiological conditions during the growth of plants, which could not be achieved due to non-availability of controlled condition growth room.

(c) The selfed and OP F_2, F_3 and BC_1 F_2 seeds from the single plants of the F_1's, F_2's and backcross generations, that were grown at Gual Pahari during October 1994 to March 1995, were harvested separately.

(d) The F_2 seeds of the crosses with low erucic acid donors were analysed during summer of 1995 and plants having <2% erucic acid were selected (Table-8). The single plant progenies of selected low erucic acid plants was grown in the field for collection of selfed seeds.

(e) The F_3 and BC_1 F_2 seeds were analysed for their glucosinolate content through HPLC to select seeds having less than 30 μm glucosinolate/g of defatted meal (Table 9).

(f) A few F_3 populations from some of the selected crosses were raised at FRL, Leh during off-season for advancement of generation. The remaining F_3 populations and some of the advanced F_4 populations were grown at Gual Pahari.

(g) From a unique three way cross (Varuna x Zem-1) x BJ-1058, attempted during 1994 summer, the F_1 seeds were harvested to grow the F_1 population and F_2 seeds were collected during Rabi 1994-95. A large number of F_2 population was grown during summer of 1995 to collect self and OP seeds from individual plants. The F_3 seeds were analysed for their glucosinolate content by HPLC. The selfed F_3 seeds of selected plants having less than 30 μm glucosinolate/g of defatted meal were subjected to half seed analysis for selection of plants having low erucic acid. The double low plants of *B. juncea,* thus selected, were grown from the remaining half seed and were selfed for collection of seeds.

(h) The low glucosinolate lines of *B. napus*, derived from TERI selections x Regent/Shiralee selected for having less than 30 μm glucosinolate/g of defatted meal, were also analysed through half seed technique to select plants/lines having less than 2% erucic acid. The double low plants/lines of *B. napus* were selfed for collection of seeds.

April 1996 - March 1997

(a) The selfed and OP seeds from low erucic, low glucosinolate and double low plants of *B. juncea* and *B. napus* were harvested separately.

(b) The selfed seeds from the low erucic acid lines were analysed to reconfirm the fatty acids profile. The agronomical data collected from replicated yield trial of low erucic acid *B. juncea* and *B. napus* lines was evaluated to select superior quality strains.

(c) The single plant seeds harvested from the individual low glucosinolate F_3/F_4 plants were analyzed and it was observed that the plants were still segregating for the glucosinolate content. It is probably due to the involvement of multiple recessive genes and the plants would be required

to be selfed and analyzed on individual plant basis till F_6 or F_7 generation, for the low glucosinolate character to get stabilized (personal communication, Dr. R. K. Downey, Agriculture and Agrifood Canada Research Station)

(d) The F_1 seeds from fresh crosses made between the selected low erucic and low glucosinolate lines for combining the double low character were harvested. The F_1 seeds were multiplied during the off-season to collect F_2 seeds.

(e) The F_4 and F_5 single plant progenies of low glucosinolate selections were grown in the field. Selfed and OP seeds were collected from individual plants.

(f) The F_2 population of the new crosses for double low (low glucosinolate x low erucic acid, as obtained in `d' above) was raised. A large number of plants were bagged. The F_3 selfed and OP seeds were collected.

(g) The single plant progenies of double low *B. juncea* and *B. napus* plants were grown in the field and selfed and OP seeds from individual plants have been collected.

pril 1997 - March 1998

(a) Director, NRC R&M was approached to obtain germplasm lines for satisfactory standardization of NIR for estimation of glucosinolate and oil/moisture content. The 50 germplasm lines supplied, having variable oil contents, were sown at TERI's field station, Gual Pahari, Gurgaon. The plants were selfed to obtain large quantities of seeds to be utilized for NIR standardization. The data on the glucosinolate, oil and moisture contents of the germplasm analysed for NIR standardization is given in Table 10.

(b) The selfed and OP F_3 seeds from the new double low crosses (obtained from low glucosinolate x low erucic acid) and F_4/F_5 low glucosinolate individual plants were harvested.

(c) The harvested F_5/F_6 seeds of low glucosinolate plants and from individual plants of double low *B. juncea* and *B. napus* lines were analysed. They were also characterised for their fatty acid contents (Table 11).

(d) The self seeds from the low glucosinolate lines and selected low erucic acid strains were multiplied during off-season (May to September 1997) at FRL, Leh.

(e) The replicated yield trial data was evaluated for quality and agronomical traits of the advance *B. juncea* (Table 12) and *B. napus* (Table 13) strains. Accordingly superior strains of low erucic acid *B. juncea* and *B. napus*

based on agronomic performance in replicated yield trial, were entered in AICRP on R&M trials for multilocation testing.

(f) The single plant progenies of low glucosinolate selections and selected double low plants were sown in the field during Rabi 1997-98. The plants were bagged for collection of self and OP seeds from the individual plants.

(g) Simultaneously the OP seeds of the selected low erucic acid/low glucosinolate/double low plants were sown for evaluation of their agronomic performance.

(h) The low erucic acid strains of *B. juncea* and *B. napus*, entered in the AICRP R&M for multilocation testing, were also tested at high density for their agronomic performance and yield evaluation to select better plant types.

pril 1998 - March 1999

(a) The NIR was standardized satisfactorily for oil and moisture contents. However, the work on NIR standardization for glucosinolates was performed with the limited stock of available germplasm seeds and can be used for screening purposes. There is a scope for further improvement in case germplasm with wide range of glucosinolate content is available.

(b) The selfed and OP seeds of individual plants from single plant progenies of low glucosinolate selections and putative double low plants were harvested separately.

(c) The agronomical characteristics were recorded for low glucosinolate/double low lines. Selections were made for better plant types.

(d) The glucosinolate content was analysed from the OP seeds of the individual plants of the single plant progenies of low glucosinolate and double low plants. The selections were made for the lines showing stability in this quality parameter. The fatty acids and glucosinolate content were analysed for the low erucic acid, low glucosinolate and double low lines.

(e) The advanced low erucic acid *B. juncea* and *B. napus* lines were evaluated for their yield potential and agronomic performance. Based on the quality status and agronomical characteristics, two zero erucic lines of *B. juncea* (derived from *B. juncea* var. Varuna x T-7): TERI(OE)M981 and TERI(OE)M982, and two zero erucic acid lines of *B. napus*: TERI(OE)R983 (TERI-18 x *B. napus* var. Regent) and TERI(OE)R984 (TERI-19 x *B. napus* var. M2) were contributed for AICRP 1998-99 for multilocation testing.

(f) The AICRP R&M multilocation data for Rabi 97-98 was discussed during the AICRP R&M meeting held Aug. 6-8 at BAU, Ranchi. The zero erucic

acid strain TERI(OE)R15 was at par in seed yield or slightly better (536-957 kg/ha) at Hisar and Morena centres as compared to the zonal check *B. juncea* variety RL1359 (536-935 kg/ha). It yielded 7.5% more than the *B. juncea* var. Vardan at Kanpur centre. However, since *B. napus* check was not included in the trials, it was decided to repeat the trial with both *B. juncea* and *B. napus* national check varieties. There was no significant difference in the yield at higher plant density, however, the best yield was recorded at the recommended spacing of 30 x 15 cm. Therefore this strain was contributed for AICRP 1998-99 for multilocation testing.

(g) Two of the low erucic acid *B. napus* lines, TERI(OE)R03 and TERI(OE)R05 performed well in IVT of Rabi 1997-98. The zero erucic acid strain TERI(OE) R03 yielded 6 to 45% more than the national check varieties of *B. napus* and *B. juncea* (GSL-1, Varuna, Kranti) in zone I. It also outyielded *B. napus* var. GSL-1 by 9.5% in zone III. The zero erucic acid strain TERI(OE) R05 yielded 10 to 51% more than the national check varieties of *B. napus* and *B. juncea* (GSL-1, Varuna, Kranti) in zone I. In zone II, it was only 1 to 5% lower yielding than *B. juncea* var. Varuna and *B. napus* var. GSL-1, and outyielded the *B. juncea* var. Kranti by 13%. Therefore, TERI(OE)R03 and TERI(OE)R05 were promoted to AVT-1 for zone I and II, and zone I and III respectively and tested during Rabi 1998-1999.

(h) The seeds from some of the selected double low plants of *B. napus* were multiplied during off-season (May to Sept. 1998) at FRL, Leh. Based on the quality and agronomical characteristics double low *B. napus* lines TERI(OO)R986 (TBN-1 x *B. napus* var. Regent) and TERI(OO)R985 (TBN-5 x *B. napus* var. Cyclone) were contributed for AICRP 1998-99 for multilocation testing (Table 14).

April1999 - September 2000

(a) The self and OP seeds from the low glucosinolate and double low individual plants grown during Rabi 1999-2000 were harvested.

(b) The agronomical characteristics were recorded to select plants/lines having better yield potential.

(c) The harvested seeds were analysed for their fatty acids and glucosinolate contents to evaluate their quality status.

(d) Accordingly the seeds of double low *B. napus* viz. TERI(OO)R9903 and low erucic acid *B. juncea* viz. TERI(OE)M9901 and TERI(OE)M9902 were

contributed to AICRP R&M for multilocation testing during Rabi 1999-2000.

(e) Two double low *B. napus* strains TERI(OO)R985 and TERI(OO)R986 were registered by NBPGR, ICAR.

(f) The yield data of two entries of low erucic acid *B. juncea* var. Varuna (viz. TERI(OE)M981 and M982), three entries of low erucic acid *B. napus* (viz. TERI(OE)R983, R984 and R15) and two entries of double low *B. napus* (viz. TERI(OO)R985 and TERI(OO)R986) tested under AICRP R&M 1998-99 was evaluated during the VI Annual meeting of AICRP R&M held on 16-17 August 1999 at Nagpur. The entry TERI(OE)R984 yielded 4.5% higher than Vaurna in Zone II. The entries TERI(OE)R15, TERI(OE)R983, TERI(OE)R984 and TERI(OO)R986 were either at par or yielded up to 26% higher than GSL-I under IVT (irrigated) zone III at Morena centre. Also the entry TERI(OE)R03, yielded 45.9% higher than GSL-I under AVT-I (Zone III) at Morena centre. However, these entries could not be promoted due to want of data from more number of centres. Accordingly in the breeding session held under the chairmanship of Dr. Basudeo Singh, Executive Director, NOVODB, a decision was taken to repeat IVT Gobhi Sarson and AVT-I Gobhi Sarson Zone III including more number of centres in each zone. Therefore, the seeds of these entries were multiplied and sent to Project Coordinator, AICRP R&M for multilocation testing during Rabi 1999-2000.

(g) The seeds of the entries TERI-M9901, TERI-M9902, TERI(OE)R983, TERI(OE)R984, TERI(OE)R15, TERI(OE)R03, TERI(OE)R05 were also sent to Dr. A.K. Chattopadhyay, Plant Pathologist, Pulses and Oilseeds Research Station for screening against club root disease of rapeseed-mustard of West Bengal. None of the lines were found resistant to club root disease.

(h) The single plant progenies of the putative stable low glucosinolate/double low plants of *B. juncea* and *B. napus* were sown at TERI field station, Gual Pahari, Gurgaon for further advancement during Rabi 1999-2000. The individual low glucosinolate and double low plants were bagged for selfing. The agronomical data was recorded.

(i) The self and OP seeds from the single plant progenies of low glucosinolate and double low plants of *B. juncea* var. Varuna were harvested and analysed for their fatty acids and glucosinolate contents. The agronomical and quality status of advanced *B. juncea* quality strains is given in Table 15.

(j) The self and OP seeds from the single plant progenies of double low *B. napus* obtained from crosses of Regent, Cyclone and Shiralee were also harvested.The OP seeds were analysed for their glucosinolate content. The self seeds of the plants having low glucosinolate were analyzed for their fatty acid content. The oil content was analyzed by NIR. The fatty acid, glucosinolate and oil content of some of the selected advanced lines of *B. napus* is given in Table 16.

(k) The analysis of OP seeds of single plants from the low glucosinolate/double low strains of *B. juncea* are showing limited segregation and have not stabilized as yet for their quality characteristics. Therefore, they need further refinement before they can be submitted for registration by NBPGR-ICAR.

(l) The multilocation trial data of the advanced quality lines contributed to AICRP R&M was disussed during the 7th Annual Meeting of the AICRP R&M held at Udaipur August 10-11, 2000. The results of quality mustard trial revealed that there is a need for agronomic improvement of the low erucic acid *B. juncea* lines (Table 17)Most of the improved quality *B. napus* lines were either at par or yielded upto 26% more than the *B. napus* (NC) var. GSL-1 and were thus promoted to AVT-I/AVT-II (Table 18, 19).

Table 1. Glucosinolate status of B. juncea lines/varieties

Line/Variety	Glucosinolate Content*
Indian Cultivars	
B. juncea cv. Varuna	High
B. juncea cv. Pusa Bold	High
B. juncea cv. Kranti	High
B. juncea cv. Bio902	High
B. juncea cv. Varuna (0)	High
TERI Selections	
T-3	Medium
T-5	Medium
T-6	Medium
T-7	High
T-11	High
T-12	High
Exotic Lines	
ZEM-1	High
Zem-83	High
BJ-1058	Low

* Determined by tes-tape method.

e 2. **Glucosinolate status of *B. napus* lines/varieties**

e/Variety	Glucosinolate content*
ian Cultivars	
iapus cv. GSL-I	High
iapus cv. ISN-706	High
:l Selections	
6	High
7	High
8	High
9	High
1	High
itic Lines	
;ent	Low
star	Low
	Low
ralee	Low
:lone	Low
Excel	Low

itermined by tes-tape method.

Table 3. Fatty acid composition of some Indian and exotic cultivars/strains of *B. juncea* and *B. napus*

		Fatty Acids (%)*					
Sl. No.	Genotype	Palmitic C 16:0	Stearic C 18:0	Oleic C 18.1	Linoleic C18:2	Linolenic C18:3	Erucic C22:1
B. juncea							
1.	Varuna	7.61	--	12.85	16.93	18.41	42.62
2.	Pusa Bold	2.23	--	12.65	20.20	20.00	51.04
3	Kranti	4.49	0.80	10.70	15.26	9.52	49.07
4.	BIO-902	3.81	1.18	12.95	17.81	17.98	44.30
5.	Zem-1	3.49	2.19	49.17	32.71	10.43	4.52
6.	Zem-2	3.41	1.85	47.02	32.79	8.59	6.21
7.	Zem-83	5.08	1.56	39.54	35.20	14.08	4.52
8.	Skorespeka	9.14	0.73	22.73	26.86	19.82	20.69
9.	Donskaja	11.66	0.95	23.76	30.29	20.41	19.13
10.	BJ-1058	2.66	1.17	9.73	14.41	22.98	48.22
11.	Varuna (0)	5.46	--	50.32	32.68	11.53	0.00
B. napus							
12.	GSL-1	5.11	1.13	21.62	14.76	20.49	36.67
13.	ISN706	5.10	1.14	23.11	18.08	22.75	29.81
14	HNS-8	3.91	--	55.67	21.92	13.37	5.13
15	Regent	4.86	1.58	57.07	21.01	11.14	4.05
16.	Westar	4.23	1.28	64.38	19.28	10.81	0.00
17	M_2	3.25	1.65	62.83	13.41	12.53	6.31
18	Shiralee	3.76	2.00	58.86	18.36	11.03	5.97
19	AC Excel	4.19	1.63	63.19	18.21	10.94	1.82
20	Cyclone	3.79	1.55	55.76	19.69	12.76	2.44

* Analysed by FID-Gas Liquid Chromatography

Table 4. Erucic acid status of *B. juncea* lines/varieties

Line/Variety	Range of erucic acid (%)*	No. of plants identified with erucic acid content*			
		<7%	7-25%	25-35%	>35%
Indian cultivars					
Varuna	40.29-51.00	--	--	--	25
Pusa Bold	47.28-55.02	--	--	--	25
Kranti	45.02-51.81	--	--	--	30
Bio 902	40.55-46.28	--	--	--	26
Varuna (0)	0.00-1.58	20	--	--	--
TERI Selections					
T-3	31.43-49.29	--	--	8	42
T-5	22.29-55.08	--	--	4	46
T-6	26.85-55.37	--	--	1	39
T-7	0.77-39.89	8	16	10	6
T-11	37.14-55.46	--	--	--	20
T-12	26 54-61.37	--	--	8	12
Exotic lines					
Zem-1	2.54-6 21	25	--	--	--
Zem-83	2 48-5 27	25	--	--	--

* Analysed by FID-Gas Liquid Chromatography.

Table 5. Erucic acid status of *B. napus* lines/varieties

Line/Variety	Range of erucic acid (%)*	No. of plants identified with erucic acid content*			
		<7%	7-25%	25-35%	>35%
Indian cultivars					
GSL-1	33.05-38.22	--	--	3	17
ISN-706	32.01-35.18	--	--	5	20
TERI Selections					
T-16	1.78-7.0	50	--	--	--
T-17	5.74-17.79	36	9	--	--
T-18	2.91-46.55	28	7	--	--
T-19	4.10-6.99	20	--	--	--
T-21	4.25-6 81	20	--	--	--
Exotic lines					
Regent	1.58-4.25	10	--	--	--
Westar	0 0 -1.58	10	--	--	--
M_2	2 54-4 58	10	--	--	--

* Analysed by FID-Gas Liquid Chromatography.

Table 6. Crosses made for transfer of quality characters

B. juncea

Genotype	Zem1	Zem83	Varuna (0)	T7	BJ 1058
Varuna	X	X	X	X	X
Pusa Bold	X	X	X	X	X
Kranti	X	X	X	--	X
T-7	--	--	--	--	X

B. napus

Genotype	Westar	Regent	AC Excel	Shiralee	Cyclone
GSL-1	X	X	X	X	X
ISN 706	X	X	X	X	X
T-18	X	X	X	X	X
T-19	X	X	X	X	X

X Crosses attempted
-- Crosses not attempted

Table 7. Erucic acid (%) status of the F_1 seeds

B. juncea				
Genotype	Zem1	Zem83	Varuna (0)	T7
Varuna	34.9	33.0	27.7(11.0)	31.5
Pusa Bold	24.4	28.7(26.1)	29.7	26.2
Kranti	34.2	27.5(30.1)	X	--
T-7	--	--	--	--
B. napus				
Genotype	Westar	Regent	AC Excel	Shiralee
GSL-1	26.1	34.7(16.6)	18.1(20.6)	26 6
ISN 706	13.6	--(9.5)	12.6	14.4
T-18	3 0	9.2	1.1(1.2)	00(00)
T-19	00	00	00	4.7

--	Crosses not attempted
Figures	% erucic acid for the crosses
Figures in parenthesis.	% erucic acid for reciprocal crosses.

Table 8. Erucic acid (%) status of F_2/F_3 and BC_1 F_2 seeds

Cross	Erucic Acid (%)	No. of plants having <2% erucic acid
Varuna x T-7	0.0-53	8
Varuna x ZEM	0 0-52	6
(Varuna x ZEM) x Varuna	23-52	none
Pusa Bold x T-7	0 0-1.06	10
Pusa Bold x ZEM	0.0-1.28	5

Table 9. Glucosinolate status of F_3 and BC_1 F_2 seeds

Crosses	Glucosinolate µm/g defatted meal	Plants having glucosinolate <30 µm/g defatted meal
B juncea		
Pusa Bold x BJ-1058	51-183	None
(Pusa Bold x BJ-1058) x Pusa Bold	54-172	None
Varuna x BJ-1058	24-128	1
BJ-1058 x Varuna	25-130	2
(Varuna x BJ-1058) x Varuna	81-176	None
(Varuna x Zem-1) x BJ-1058	16-134	5
T-7 x BJ-1058	25-113	4
(T-7 x BJ-1058) x T-7	35-104	None
B napus		
ISN-706 x Shiralee	16-120	6
T-18 x Shiralee	16-90	10
T-19 x Regent	16-120	15

Table 10: Glucosinolate, Oil and Moisture Content of Brassica Cultivars

Strain/variety	Glucosinolate µm/g defatted meal	Oil content (%)	Moisture content (%)
B. juncea			
Pusa Bold	120.87	44.44	13.90
BIO-902	130.73	44.00	7 96
ZEM	106.532	42.91	12 60
B. napus			
GSL-1	70.38	39.00	11.45
ISN-706	113 10	43.00	8 72
AC Excel	29.61	40.39	14 37
M2	27.13	41.47	6.60
B. campestris			
R-500	93.167	43 07	6.73
B. nigra			
IC-257	90.58	23.75	8.80

Table 11. Erucic acid and glucosinolate contents of `0/00' lines (F5/F6) developed at TERI

Lines	Range of glucosinolate µm/g deoiled cake	Range of erucic acid (%)	Status
B. juncea			
Varuna x T-7/ZEM	--	0.0 - 1.5	low erucic acid
Pusa Bold x T-7/ZEM	--	0.0 - 1.2	low erucic acid
T-7 x BJ-1058	10-66	0.0 - 46 0	low erucic acid/low glucosinolate
Varuna x BJ-1058	10-84	48.0 - 50.0	low glucosinolate
(Varuna x ZEM) x BJ-1058	10-55	0.0 - 44.0	double low
B napus			
ISN-706 x Shiralee*	20-42	17.0 - 30 0	low glucosinolate
T-18 x Shiralee	5-24	0 0 - 1 0	double low
T-19 x Regent	3-33	0.0 - 0 5	double low
T-18 x Cyclone	12-32	0 0 - 1 8	double low

* Agronomically very poor performing, not forwarded.

Table 12. Characteristics of *B. juncea* quality strains developed at TERI

S. No	Characters	TERI(OE) M08	TERI(OE) M21	TERI(OE) M07	TERI(OE) M10	TERI(OE) M12	Varuna (NC)
1	Plant height (cm)	179	167	177	177	158	170
2	No. of pods/plant	296	258	490	471	329	530
3	1000 seed weight(g)	3.66	3 63	2.89	2.88	2.99	4.29
4	Seed colour/size	Mustard/ medium	Mustard/ medium	Mustard/ small	Mustard yellow/ medium	Mustard/ Medium	Brown/ medium
5	Days to maturity	136	117	139	139	143	143
6	Yield (Q/ha)	18.76	18 32	17 61	18 30	15.70	20.38
7	Oil content (%)	45.6	43 7	42.8	46.1	43.0	44.4
8	Oleic acid (%)	45.0	40.6	34 47	50.0	41.37	11.42
9	Linoleic acid (%)	36.63	36.3	36.52	30.7	38.42	19.07
10	Linolenic acid (%)	13.84	15.2	16.49	10.4	11.91	10.52
11	Erucic acid (%)	0.0	0 0	0.0	0 0	0.0	48.43

Table 13. **Characteristics of *B. napus* quality strains developed at TERI**

S. No	Characters	TERI(OE) R03	TERI(OE) R15	TERI(OE) R05	TERI(OE) R09	GSL-1 (NC)
1	Plant height (cm)	140	101	88	122	165
2	No. of pods/plant	291	210	212	255	308
3	1000 seed weight (g)	3.05	3.87	3.01	2.50	3.47
4	Seed colour/size	Dark Brown/ medium	Dark brown/ medium	Brown/ medium	Brown/s mall	Brown/ Small
5	Days to maturity	136	118	141	149	153
6	Yield (Q/ha)	19.01	14.23	17.58	13.31	15.90
7	Oil content (%)	43 3	46.3	47.5	50.2	46 4
8	Oleic acid (%)	59 5	45.60	47.68	70.1	24 42
9	Linoleic acid (%)	20.9	22.50	22.73	13.3	14 67
10	Linolenic acid (%)	12.5	8.13	10.19	7.3	8.87
11	Erucic acid (%)	0 0	0.0	0 0	0 0	36 93

Table 14. The agronomical and quality characteristics of the double low *B. napus* strains

Strains	TERI(OO)R986	TERI(OO)R985	GSL-1 (NC)
Pedigree	*B. napus* line TBN-1 x *B. napus* var. Regent	*B. napus* line TBN-5 x *B. napus* var. Cyclone	
Agronomical Characteristics			
Plant height (cm)	121	174	176
No. of primary branches	6	10	8
No. of secondary branches	4	5	4
Length from base to first branching (cm)	7	41	75
No of pods/plant	218	291	155
No. of siliquae on main branch	28	47	34
Siliquae length (cm)	6 3	6.6	6.6
No. of seeds per siliquae	22	23	20
1000 seed weight (g)	2.76	3.13	2.25
Seed colour/size	Dark brown/ Medium	Dark brown/ Medium	Light brown/ Small
Seed yield/plant (g)	13.08	15.21	8 72
Days to maturity	125	143	153
Quality Characteristics			
Oleic acid (%)	48 0	57.2	21.6
Linoleic acid (%)	32.7	22 3	14.8
Linolenic acid (%)	12.9	10.5	20 5
Erucic acid (%)	0 0	0 0	36.7
Glucosinolate (μm/g oil free meal)	15 3	12.2	77.2

Table 15. **The important agronomic and quality characteristics of the low erucic acid mustard (LEM), low glucosinolate mustard (LGM) and double low mustard (DLM) strains of *B. juncea* var. Varuna**

Characteristics/Strains	LEM	LGM	DLM	Varuna*
Plant height (cm)	195-205	170-197	198-242	190-200
Days to maturity	135-140	132-140	145-152	140
Seed yield (g/plant)	24-29	14-19	15-20	25-32
Oleic acid (%)	30-44	12-15	35-44	12.8
Linoleic acid (%)	28-31	17-24	43-48	16.9
Linolenic acid (%)	15-22	11-13	6-10	18.4
Erucic acid (%)	0-1.5	39-44	0.0	42.6
Glucosinolate (µm/g oil free meal)	127	9-25	10-27	163

* National check variety *B. juncea* var. Varuna

Table 16: Fatty Acid Composition, Glucosinolate and Oil Content of *B. napus* strains being evaluated under AICRP R&M

Genotype	Fatty Acid Composition (%)						Oil Content (%)	Aliphatic GSL µmole/g defatted meal
	Palmitic 16:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Eicosenoic 20:1	Erucic 22:1		
TERI(OE)R-03	2.87	56.98	18.44	6.61	11.53	1.56	32.59	98
TERI(OE)R-05	3.08	52.65	21.25	7.81	10.37	1.25	34.52	93
TERI(OE)R-09	3 42	70.1	13.3	7.3	2.42	0.0	50 2	76
TERI(OE)R-15	4.30	41.10	25.87	10 55	14.08	4.06	42.03	80
TERI(OE)R-983	4.70	50.14	27.77	10 40	6.96	0.0	37.01	111
TERI(OE)R-984	2.53	67.48	21 11	7.63	0.0	1.26	39.48	73
TERI(OO)R-985	9.10	54.48	20.41	10.23	3.90	1.74	40.63	17
TERI(OO)R-986	3 74	54.39	26.74	14 60	0.51	0.0	38.18	28
TERI -R 9903	3.91	56 29	23.95	10.59	4 98	0.0	38.99	32
GSL-1 (NC)	4.25	16.41	17 07	8.72	13 28	39.82	42 71	74

NC: National Check

Table 17. Comparative Yield (%) of *B. juncea* quality strains with respect to National Checks (NC) and Zonal Checks (ZC) varieties

IVT-I - Quality Mustard : Zone II

Strains	1997-1998			1998-1999			1999-2000		
	Kranti (NC-1)	Varuna (NC-2)	RL-1359 (ZC)	Kranti (NC-1)	Varuna (NC-2)	RL-1359 (ZC)	Kranti (NC-1)	Varuna (NC-2)	RL-1359 (ZC)
TERI(OE) M-21-1	55.99	57.86	54.67	-	-	-	-	-	-
TERI(OE) M-12	60 78	62 8	59.34	-	-	-	-	-	-
TERI(OE) M-10	46 90	48 46	45.78	-	-	-	-	-	-
TERI(OE) M-07	64.08	66.22	62 56	-	-	-	-	-	-
TERI(OE) M-981	-	-	-	92.15	90.99	77.94	-	-	-
TERI(OE) M-982	-	-	-	88.71	87.59	75.03	-	-	-
TERI(OE) M-9901 (M-981)	-	-	-	-	-	-	75.34	84.15	71.63
TERI(OE) M-9902	-	-	-	-	-	-	61.30	68.47	58.29

IVT-I - Quality Mustard : Zone III

Strains	1997-1998			1998-1999			1999-2000		
	Kranti (NC-1)	Varuna (NC-2)	RL-1359 (ZC)	Kranti (NC-1)	Varuna (NC-2)	Rohini (ZC)	Kranti (NC-1)	Varuna (NC-2)	Rohini (ZC)
TERI(OE) M-21-1	31.51	51 79	23 46	-	-	-	-	-	-
TERI(OE) M-12	20 23	33 24	15 06	-	-	-	-	-	-
TERI(OE) M-10	18 21	29 92	13 55	-	-	-	-	-	-
TERI(OE) M-07	33 30	54.73	24 79	-	-	-	-	-	-
TERI(OE) M-981	-	-	-	79.36	92.61	91.39	-	-	-
TERI(OE) M-982	-	-	-	70.88	82 71	81.63	-	-	-
TERI(OE) M-9901 (M-981)	-	-	-	-	-	-	87 79	80.92	72.25
TERI(OE) M-9902	-	-	-	-	-	-	66.44	61.24	54.68

Table 18. Comparative Yield (%) of *B. napus* quality strains, promoted to AVT-I, with respect to National Check (NC) varieties

IVT-I - Gobhi Sarson : Zone I

Strains	1998-1999			1999-2000		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-15	71.67	80.70	53.37	68.68	87.83	62.67
TERI(OE) R-983	78.83	88.76	58.70	66.83	85.46	60.98
TERI(OE) R-984	93.24	105.00	69.44	78.95	100.96	72.04
TERI(OO) R-985	104.83	118.04	78.07	99.83	127.66	91.09
TERI(OO) R-986	67.84	76.39	50.52	73.06	93.43	66.66
TERI(R) 9903				80.38	102.79	73.34

IVT-I - Gobhi Sarson : Zone II

Strains	1998-1999			1999-2000		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-15	66.11	73.06	65.97	56.73	64.07	70.00
TERI(OE) R-983	78.56	86.81	78.38	59.12	66.78	79.95
TERI(OE) R-984	94.57	104.5	94.35	71.76	81.05	88.50
TERI(OO) R-985	88.97	98.31	88.76	80.87	91.33	99.79
TERI(OO) R-986	77.82	86.00	77.65	54.85	61.95	67.68
TERI(R) 9903				64.66	73.03	79.78

IVT-I - Gobhi Sarson : Zone III

Strains	1998-1999			1999-2000		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-15	58.78	61.26	109.04	73.44	92.50	109.97
TERI(OE) R-983	53.65	55.92	99.54	82.38	103.77	123.37
TERI(OE) R-984	68.29	71.17	126.69	84.15	105.99	126.01
TERI(OO) R-985	48.19	50.22	89.41	63.44	78.71	95.01
TERI(OO) R-986	65.02	67.76	120.63	82.29	103.65	123.23
TERI(R) 9903				68.58	86.39	102.7

Table 19. **Comparative Yield (%) of *B. napus* quality strains, promoted to AVT-II, with respect to National Check (NC) varieties**

Zone I

Strains	1997-1998 (IVT-1)			1998-1999 (AVT-1)			1999-2000 (AVT-1)		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-03	145.60	143.8	106.8	85.10	111.89	71.50	-	-	-
TERI(OE) R-05	151.0	149.2	110.8	70.47	92.66	59.21	-	-	-
TERI(OE) R-09	106.08	104.8	77.85	-	-	-	-	-	-

Zone II

Strains	1997-1998 (IVT-1)			1998-1999 (AVT-1)			1999-2000 (AVT-1)		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-03	56.59	74.77	90.25	-	-	-	-	-	-
TERI(OE) R-05	90.62	119.72	144.5	60.63	78.46	67.22	-	-	-
TERI(OE) R-09	65.50	86.50	104.42	-	-	-	-	-	-

Zone III

Strains	1997-1998 (IVT-1)			1998-1999 (AVT-1)			1999-2000 (AVT-1)		
	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)	Kranti (NC-1)	Varuna (NC-2)	GSL-1 (NC)
TERI(OE) R-03	69.32	95.89	109.53	59.40	68.72	145.94	59.57	73.58	105.58
TERI(OE) R-05	49.59	68.59	78.35	-	-	-	-	-	-
TERI(OE) R-09	53.40	73.83	84.32	-	-	-	-	-	-

Recommendations and Follow-up Actions

Follow -up action on the recommendations of the Ist and IInd review committee

Follow up action on the recommendations of the 1st and 2nd review committee, according to the approved minutes is as follows :-

	Recommendation	Action Taken
(1.1)	Allelic relationship of genes of TERI lines with that of exotic lines should be determined	A number of crosses involving TERI lines (T-7, T-18, T-19) and Indian and Exotic released varieties of *B. juncea* i.e. Varuna, Pusa Bold, Kranti, Zem-1, Zem-83 and of *B. napus* ISN-706, GSL-1, AC Excel, Shiralee, Regent, Westar were made during 1993-94 and F1 seeds were harvested. For finding out the allelic relationship we need to self these F1 plants individually to obtain selfed F2 seeds, grow the F2 population and analyse the segregating populations. Considering only one TERI line (T-7) crossed with one exotic (Zem-83) and three Indian (Pusa Bold, Varuna, Kranti) cultivars, we would need to analyse at least 500 plants from each of the four crosses. Also, in the backcross generation at least 100 plants of each cross need to be analysed. Similarly, taking two TERI lines in *B. napus* and four exotic and two Indian *B. napus* cultivar we would need to analyze F2 and backcross population of 500 and 100 plants each for 12 different crosses. We agree that it will be of great academic interest to find the allelic relationship, however, at the same time it is beyond the scope and objective of our project i.e "To develop '00' varieties of rapeseed-mustard" We regret that due to the constraint of manpower, time and resources it may not be possible for us to carry out the exercise of finding out the allelic relationship. The F1 seeds can be given to the interested scientists through NOVODB/ICAR who can take it up as a separate programme/project. We would prefer to concentrate on the main objective of the project i.e. developing '00' lines of rapeseed mustard from the existing and available germplasm.

	Recommendation	Action Taken
(1.2)	As objective is to transfer '00' genes in improved/released varieties, the recurrent parents may not be confined to just one plant	The *B. juncea* and *B. napus* lines developed at TERI, and being used in the programme, are already adapted to Indian conditions. In addition, a number of crosses have been made with improved varieties like Varuna, Pusa Bold, Kranti, ISN-706, GSL-1, for transfer of '00' genes from exotic germplasm to the Indian released varieties. Also, as suggested, the backcrossing programme is being continued.
(1.3)	Period to develop '00' varieties should be reduced by modifying the hybridization/selection schedule	Biotechnological approach consisting of half seed analysis for selection of zero erucic acid plants has been incorporated. During the current season (1994-95) we are also trying to culture the anthers from the F1 plants, which if successful, would help for early selection of the desired plants. Through conventional breeding it generally takes at least 6-7 years to develop a strain and 8-10 years to develop a variety. However, we are quite hopeful that we may be able to get '00' lines in 5 years by modification of the programme as mentioned above.
(1.4)	Approved technical programme of work should invariably be given in the progress report	The approved technical programme was given during the second review meeting and has also been included this time.
(1.5)	There is need to find out the reasons for getting low 22:1 and low glucosinolate content in progenies of parents having high erucic acid and high glucosinolate content	It is very important to analyze and determine the quality level of the original parents for finding out the reasons for getting low 22:1 in progenies involving high erucic acid At the time of initiation of Brassica Biotechnology Programme at TERI about nine years back, we did not have the facility for quality analysis and the germplasm obtained from IARI was used as such The initial analysis was performed at Agriculture Canada Research Station, Saskatoon during 1991-92 with the help of Dr. R.K. Downey (Former Head - Oilseed Section) and Dr. J.P. Raney (Senior Scientist - In charge, Oil quality lab). Our recent attempts to get the seeds of the parents involved in the original crosses for doing the quality analysis were not successful. We have discussed in details with Dr. Downey and it follows that since exotic zero erucic and '00' material has remained in India for a very

	Recommendation	Action Taken
		long time, there could be two possibilites: The germplasm used in initial crosses was already outcrossed with exotic zero/00 material. The outcrossing may have occurred at a later stage during the course of the work. Since it is very difficult to find out and determine the exact answer to the above mentioned possibilities, we would submit that we may concentrate on utilizing the available better quality material in best possible ways for development of '00' varieties suitable to grow under Indian conditions. However, as mentioned in item no.1.1, the seeds of F1 crosses available with us can be given to the interested scientists, for determining the allelic relationship and the reasons mentioned under item no. 1.5 for academic research.
(1.6)	Since the genotype of the embryo controls the erucic acid concentration, therefore, it is necessary to follow the half seed method. This will reduce one year of experimentation	Half seed technique has been incorporated for selection of zero erucic acid plant.
2.	Asstt. Director General (OS) informed that Dr. A.S. Khalatkar of Nagpur University has developed zero erucic acid strains i e Shiva and Nihal Seeds of these two types would be made available to TERI through ADG (OS)	We will incorporate these strains in our programme if seeds are made available.
3	It was noted that the data on glucosinolate was not quantified, therefore, the requirement of NIR was considered by the aforesaid review committee	Order for NIR has been placed and is likely to arrive by April 1995 which would help in quick quantitative estimation of glucosinolates. HPLC was installed during March 1994. A suitable method was deviced for separation of individual glucosinolate and their exact quantification. However, work is in progress for further standardization to get better quantification.
4.	Committee also suggested that being a research project, review may be made yearly instead of half yearly basis and accordingly next review is proposed in February 1995, when the results of the current rabi and off-season trials (1994) will be available	The third review meeting is due in February 1995. A joint review meeting for all the centres working on '00' varieties is being held on Feb. 20th 1995 at NOVODB.

	Recommendation	Action Taken
5.	It was suggested, that the project scientists may be acquainted with the work being going on at the other centres like HAU, Hisar, PAU, Ludhiana, and Botany Department, Nagpur University	TERI scientists participated in the First Annual Rapeseed-Mustard Research Worker's Group meeting, August 20-22, 1994, held at Gwalior and interacted with the scientists present from PAU, Ludhiana, CSAU, Kanpur and NDDB, Anand.
6.	Committee found that there is no close coordination among the various organizations for sharing of knowledge and expertise on the development of '00' varieties and suggested for a joint review meeting to be organised by ICAR for close coordination and interaction among all the five centres working on development of '00' varieties of rapeseed and mustard	A joint review meeting is being held on Feb. 20th, 1995 at NOVODB.

Follow -up action on the recommendations of the IIIrd review committee

Follow up action on the recommendations of the 3rd review committee, according to the approved minutes (No. 32-2/NOVOD/95/379-92 dated 5.5.95) is as follows :-

	Recommendation	Action Taken
(1)	For efficient coordination a network on '00' types should be created under the aegis of ICAR comprising scientists from ICAR, NOVOD Board, AICORPO and SEA. This network should prepare a time bound action plan and monitor the progress regularly.	Recommendation nos. 1, 3, 5, 6, 7, and 8 are being persued by ICAR/NOVODB, Ministry of Agriculture, Govt. of India. A proposal has been submitted to ICAR for the Cooperative National Network on Oilseed *B. juncea* quality and is under consideration.
(3)	Areas should be identified for cultivation of such materials and initial evaluation may be undertaken in Rajasthan.	
(5)	Central facility for testing of material especially for erucic acid and glucosinolates may be created for optimum benefits of network approach	
(6)	Better facilities for off-season multiplications for rapeseed and mustard may be strengthened at one place only by the ICAR	
(7)	Provision of controlled growth chambers may be considered on need basis under individual projects	
(8)	Until '00' type is developed, possibilities of developing technology to remove the glucosinolate from the rapeseed meal may be explored by NOVODB	
(2)	As a short term measure more efforts should be made on development of varieties with the low glucosinolates, whereas development of '00' varieties should be a long term approach	Keeping in view the recommendation no 2 and 4, the emphasis was laid on developing low glucosinolate lines in *B. juncea*. As a result, low glucosinolate lines have been identified in the background of *B. juncea* var. Varuna and strain T-7. The F3 and F4 plants having less than 30 µm glucosinolate/g of defatted meal are being grown in the field. After collection of selfed seeds, the single plant progenies would be grown in the subsequent generation for evaluation of their agronomic performance Preliminary results indicate some of the lines to be at par with *B. juncea* var. Varuna for 50% flowering.
(4)	As *B. juncea* is largely grown in India, major thrust should be given on *B. juncea*. However in hand programmes on development of '00' varieties in *B. napus* may continue	

Follow -up action on the recommendations of the IVth review committee

Follow up action on the recommendations of the 4th review committee, according to the approved minutes (No. 32-2/NOVOD/95 dated 25.3.96) is as follows :-

	Recommendation	Action Taken
(i)	To protect the interest of plant breeders/geneticists a strategy is being chalked out by the ICAR for registration of parental material and valuable genetic stocks developed by research Institutions/breeders. TERI should get in touch with ADG(OP) for necessary follow-up for registration of the valuable single low and double low lines of *B. napus* and *B. juncea* developed by it	Discussions were held with Dr. E.A. Siddiq, DDG (CS), ICAR, Dr. N.B. Singh, ADG (OP), ICAR, Dr. S.M. Ilyas, ADG(IPR), ICAR and Dr. K.P.S. Chandel, Director, NBPGR regarding registration of quality lines developed at TERI. Accordingly an application was submitted to DDG, ICAR and an application along with seed material of four zero erucic acid strains of *B. juncea* and *B. napus* [TERI(OE)M08, TERI(OE)M21, TERI(OE)R03 and TERI(OE)R15] have been submitted to Director, NBPGR.
(ii)	Material developed by TERI should be entered in All India coordinated trial for their evaluation	Four zero erucic acid strains have been submitted to Dr. P.R. Kumar, Director, NRC R&M, Bharatpur, for inclusion in the AICORP trial on R&M.
(iii)	An UNDP project under preparation envisages on farm trials of `0-0' varieties through ICAR, TERI may make available their lines for such trials while entering them into All India Coordinated trials.	The seeds of four zero erucic acid rapeseed-mustard strains have been given to Dr. P.R. Kumar, Director, NRC R&M, Bharatpur, for on-farm trial under the UNDP-ICAR programme. Ten quality rapeseed-mustard trials have been laid down at different village sites in the district Gurgaon Dr. N.B. Singh, ADG(OP), ICAR and Dr. J.P. Singh, Secretary, NOVODB, visited two of these trials on 2.1.97. However, no sanction/financial assistance under the UNDP-ICAR programme has been released as yet. We, therefore, request the board to kindly provide financial assistance towards the expenditure incurred, as an additional fund over and above the sanctioned outlay for 96-97, being a subsidiary programme.

	Recommendation	Action Taken
(iv)	The request of TERI for reappropriation of funds should be considered favourably by the Board.	We are grateful to the board for having considered our request favourably. A revised year-wise (Dec. 1992 to November 1997) and itemwise allocation of funds for reappropriation has been submitted to NOVODB. We further request the board to kindly convey the formal approval in this regard.
(v)	Secretary, NOVODB, who is basically a breeder having adequate experience in this field, may extend his field guidance to TERI in identification of suitable material for incorporation in AICORP and on-farm trials.	Dr. J. P. Singh, Secretary, NOVODB visited our field station and has provided his expert comments and advice on selection of strains/lines. Accordingly four zero erucic acid strains [TERI(OE)M08, TERI(OE)M21, TERI(OE)R03 and TERI(OE)R15] have been incorporated in AICORP and UNDP-ICAR on-farm trials
(vi)	Request of TERI may also be considered for provision of Controlled Condition Growth Chamber/Greenhouse to maintain the genetic purity of the valuable material developed.	We request the NOVODB/ICAR once again for making provision of greenhouse for maintaining the genetic purity of the valuable quality material developed at TERI.

Follow up action on the recommendations of the Vth review committee

According to the approved minutes (No. 32-2/NOVOD/97-389-99 dated 21.3.97), the action taken on the recommendations of the Vth review held in February 1997, is as follows:

Recommendation	Action taken
(a) More agronomical trials be laid out by TERI to determine optimum plant population, date of sowing and application of input for higher economic returns from varied plant types developed under the programme	The low erucic acid *B. juncea* and *B. napus* strains, developed at TERI and selected after agronomical/yield evaluation, have been entered in the AICRP on R&M for multilocation testing. Simultaneously some of the strains are being evaluated at different plant densities and agricultural inputs, both under AICRP and at TERI's field station, Gual Pahari, Gurgaon.
(b) Director, NRC R&M shall help in the identification of suitable areas for laying out field trials for low erucic and low glucosinolate lines of *B. juncea* and *B napus*	The seeds of low erucic acid *B. juncea* strain TERI(OE)M21 and *B. napus* strain TERI(OE)R03 are being tested at farmers field through Director, NRC R&M
(c) ADG(OP) and Secretary, NOVOD Board having rich experience in this area shall continue to provide technical input for improving breeding/selection methodology at field level	Dr. N.B. Singh, ADG(OP), ICAR and Dr. J.P. Singh, Secretary, NOVODB, visited TERI's field station, Gual Pahari on 22.3.97 and provided their valuable suggestions for selection etc.
(d) TERI shall approach NBPGR and NRC R&M for collection of needed germplasm for accelerated breeding research and quick standardisation of NIR	Fifty germplasm lines have been obtained through Director, NRC R&M and are being selfed for multiplication of seeds We also requested Dierctor, NBPGR to obtain the low glucosinolate donor line BJ-1058 from Agriculture Canada Research Station, Canada.
(e) ADG(FC-I), ICAR shall do the needful for implementing the registration of germplasm	The proforma for registration was obtained from ADG(FC-I)and NBPGR. Four fresh proposals were submitted for registration. As per the suggestions of the Germplasm Committee for Registration, ICAR, the revised proposals have been submitted for registration
(f) To create and to promote low erucic and low glucosinolate lines, appropriate measures may be taken by NOVOD Board in consultation with TMOP. A separate meeting may be convened inviting representative of oil industry, NDDB, TMOP, ICAR, NOVOD and TERI in this regard.	Action to be taken by NOVODB/ICAR.
(g) The request of TERI for reappropriation of funds alongwith extension of the programme upto March 2000, when the project is expected to end to a logical	A review meeting was held at Krishi Bhawan under the Chairmanship of Shri K Rajan, Secretary (A&C) and Chairman, Managing Committee, NOVODB on

Recommendation	Action taken
conclusion be considered for approval.	10.7.97. Based on the progress made so far, the project has been accepted for extension till March 2000 through their letter No. 32-2(7)/NOVODB/ 97-7063-65 dated 20.10.97.
(h) TERI may explore the possibilities for installing growth chamber/green house from their own resources.	The possibility of procuring the growth chamber/green house is being considered under the ICAR-NDDB collaborative network project on Improvement of Quality of Oilseed Brassicas.
(i) The Council may examine the suggestion to consider and include TERI as one of the co-operating centre, under the All India Coordinated Research Project on Rapeseed & Mustard.	A proposal has been submitted through Dr. R.S. Paroda, DG, ICAR to Director NRC R&M for consideration of TERI as a centre of ICAR under IX plan.

Follow up action on the recommendations of the VIth review committee

According to the approved minutes (No. 32-2(7)/NOVOD/98/20793-20801 dated 16.3.98), the action taken on the recommendations of the VIth review held in February 1998, is as follows:

Recommendation	Action taken
(a) TERI may engage an experienced breeder as a consultant in case the post of breeder is not filled up immediately, to strengthen the programme.	As per discussions held with Dr. Basudeo Singh, Executive Director, NOVOD and Dr. J.P. singh, Secretary, NOVODB, Prof. B. Rai, BHU, Varanasi and Prof. S.S. Bhadouria, JNKVV, Morena, having rich experience in Brassica breeding, were approached with a request to join the expert group of consultants to strengthen the programme. Useful discussions were held during their visit to TERI in January/ February 1999.
(b) In view of the scaling up of the project expected outcome, the request of TERI may be examined favourably by the Board for enhancing the outlays under chemical/consumable TERI should consider to identify the required type of land to grow and test the material under required agronomical farm situations, where rapeseed and mustard is grown. The land may be hired by TERI on lease basis for above purposes. Cost of the lease may be booked under consumable.	We appreciate that while considering the total expenditure of Rs. 75.42 lakhs agains the sanction of Rs. 77.06 lakhs, for the period upto March 1998, the NOVOD Board has very kindly agreed to reappropriate the balance of Rs. 1.64 lakhs under the chemical and travel during 1998-99. As discussed with Dr. Basudeo Singh, ED, NOVOD and Dr. J.P. Singh, Secretary, NOVODB, the quality material is at an advanced stage requiring constant supervision and selection. For the sake of safety and security of this valuable material, and due to logistic reasons, presently it may not be advisable to grow it at farmers field. At present, after confirming the quality status, the potential lines are being selected based on their comparative performance which may be utilized for selection for further advancement.
(c) The promising materials should be evaluated under specific plant geometric trials in collaboration with AICRP on R&M	The promising material are being included in the agronomical trial for testing under different density of plant populations in collaboration with AICRP on R&M
(d) The material developed specially for *Brassica juncea* which is a priority crop for development of `00' varieties may be suitably registered and provided to other institutions for further use of the material in their breeding programmes, which appears to be the objective of the project.	The two double low lines of *B. napus* TERI(00)R985 and TERI(00)R986 were submitted for registration by NBPGR-ICAR in August 1998 and is under consideration. The low glucosinolate/double low strains of *B. juncea* may be applied for registration after the results of Rabi 1998-99 are available.
(e) NOVOD should take up the matter with TMOP/Govt.	Being pursued by NOVODB

Recommendation	Action taken
for creating an environment for quality consciousness and payment of remunerative price to the farmers on the basis of oil content and quality parameters.	

Follow up action on the recommendations of the VIIth review meeting

According to the approved minutes (No. 32-2(8)/NOVOD/99/93-101 dated 19.4.99), the action taken on the recommendations of the VIIth review held in March 1999, is as follows:

Recommendation	Action taken
(a) More lines/strains with low erucic and low glucosinolate and with double low characteristics should be incorporated in AICRP for assessing their suitability and aboptability for commercial cultivation.	The following low erucic acid/double low strains of *B. juncea* and *B. napus* are being evaluated under AICRP R&M during Rabi 1999-2000. (i) Low erucic acid *B. juncea* TERI M9901 *B. juncea* var. Varuna x T-7 TERI M9902 *B. juncea* var. Pusa bold x T-7 (ii) Low erucic acid B. napus TERI(OE)R983 TERI-18 x B. napus var. Regent TERI(OE)R984 TERI-19 x B. napus var. M2 TERI(OE)R15 (*B. napus* x *Raphanobrassica*) x *B. napus* TERI(OE)R03 (*B. napus* x *Raphanobrassica*) x *B. napus* TERI(OE)R05 (*B. napus* x *Raphanobrassica*) x *B. napus* (iii) Double low *B. napus* TERI(OO)R985 TBN-1 x *B. napus* var. Regent TERI(OO)R986 TBN-5 x *B. napus* var. Cyclone TERI(OO)R9903 TBN-1 x *B. napus* var. Cyclone
(b) Concerted efforts should be made to analyze the more seed samples with desired characters and to purify andmultiply the stable lines for further testing and multiplication	Appropriate analysis has been performed.The seeds of the lines submitted to AICRP are being multiplied.
(c) The material developed under the project should be registered with NBPGR (ICAR)	The following quality strains have been registered at NBPGR-ICAR SWARNA-TERI(OE) M21-INGR 98001; Zero erucic acid, yellow seeded, early maturing (117 days), *B. juncea* PHAGUNI-TERI(OE)R03-INGR 98002; Zero erucic acid, early maturing (136 days), *B. napus* SHYAMALI-TERI(OE)R09-INGR 98005; Zero erucic acid, high oleic (70.1%), high oil content (50.2%), *B napus* GAURAV-TERI(OO)R985-INGR 99007; Zero erucic acid, low glucosinolate (15.3 µm/g oil free meal), early maturing (125 days), dwarf double low rapeseed GARIMA-TERI(OO)R986-INGR 99008; Zero erucic acid, low glucosinolate (12.2 µm/g oil free meal), high oleic acid (57%), double low rapeseed.

Recommendation	Action taken
(d) The request of TERI was examined and it was agreed that Board should enhance the salaries as per ICAR norms for revision of the salaries of Research Staff engaged on contractual basis in the project.	We are thankful to the Board for enhancing the salaries as per ICAR norms
(e) The request of TERI for re-appropriation of funds from saving may be examined favourably by Board on the basis of justified requirement. Accordingly Board may consider 20% cost escalation under Travel and chemicals head during 1998-1999 and 1999-2000 over and above the average utilization under the head during previous years.	We are thankful to the Board for recurring grant reappropriation.
(f) The expert group appreciated the progress made under this project during 1998-99 and recommended the project for continuation during 1999-2000 to reach the project at a logical conclusion.	The project work is being continued as recommended.

Follow up action on the recommendations of the VIIIth review meeting

According to the approved minutes (No. 32-2(9)/NOVOD/99/1479-51 dated 11.1.2000), the action taken on the recommendations of the VIIIth review held on 30th December 1999, is as follows:

Recommendation	*Action taken*
(a) The *B. napus* material which is in advance should be promoted for its cultivation on commercial scale.	Three zero erucic and three double low strains of *B. napus* are under different stages of multilocation testing under AICRP R&M for their yield evaluation.
(b) The group recognized the good work done by TERI i.e. transferring of '0' erucic and '0' glucosinolate character in the background of *B. juncea*. The development of potential donor is a right direction for subsequent breeding for development of suitable canola type of varieties.	The work will be undertaken to combine the genepool for quality with good agronomic characteristics.
(c) All the lines should be registered with NBPGR and may be supplied to the breeders through Director, NRC R&M for its use	The five enhanced quality rapeseed mustard germplasm lines have been registered with NBPGR, ICAR.
(d) The NOVOD Board may consider the extension of the project for a period of 6 months i.e upto Sept. 30, 2000 The cost estimate under the various components may be considered on the basis of ongoing utilization of funds under these heads	We are grateful to NOVODB for the needful.

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Annexures

Annexure 1

Sanctioned Budget

(December 1992 to November 1997)

(Rs. in lakh)

Item	1992-93 (4 months) Dec. to March	93-94	94-95	95-96	96-97	97-98 April 97 to Nov. 97 (8 months)	Total
Salary/Wages	0.83	2.73	3.00	3.30	3.63	2.43	15.92
Chemical/ Consumables	2.40	2.64	2.90	3.19	3.51	--	14.64
Travel	0.75	0.82	0.91	1.00	1.10	--	4.58
Equipments	20.60	__	12.00	--	--	--	32.60
	24.58	6.19	18.81	7.49	8.24	2.43	67.74
Contingencies @10%							6.77
Grand Total							74.51

Sanctioned budget for the extension period

(December 1997 to March 2000)

(Rs. in lakh)

	December 97 - March 98	April 98 - March 99	April 99 - March 2000
1) Salary/wages	1.22	4.03	4.43
2) Chemical/consumables	0.80	2.64	2.90
3) Travel	0.30	0.99	1.10
4) Contingency	0.23	0.77	0.84
TOTAL	2.55	8.43	9.27
GRAND TOTAL			20.25

Annexure 2

Sanctioned and Revised Sanctioned Budget for the period 1998-1999

(Rs. in lakh)

Component	Sanctioned 1998-99	Re-appropriated budget 1998-99
Salary/wages	4.03	4.16
Chemical/consumables	2.64	3.57
Travel	0.99	1.04
Contingency	0.77	0.76
Total	8.43	9.53

Sanctioned and Revised Sanctioned Budget for the period 1999-2000

(Rs. in lakh)

Component	Sanctioned 1998-99	Re-appropriated budget 1999-2000
Salary/wages	4 43	4.40
Chemical/consumables	2.90	3.72
Travel	1.10	1.22
Contingency	0.84	0.43
Total	9.27	9.77

Sanctioned budget for the Extension Period (April-September 2000)

(Rs. in lakh)

Component	Sanctioned Outlay
Salary/wages	1.42
Chemical/consumables	1.86
Travel	0.20
Contingency	0.35
Total	3.83

Annexure 3

TATA ENERGY RESEARCH INSTITUTE
NEW DELHI

Statement of Expenses for the project entitled "Development of Double Zero Varieties of Rapseed Mustard"

S No	Particulars	Revised Budget	Expenses 1 12 92 to 31.3 93	Expenses 93-94	Expenses 94-95	Expenses 95-96	Expenses 96-97	Expenses 97-98	Expenses 98-99	Expenses 99-2000	Actual Expenses 1.4 2000 to 30.9 2000	Total
1	Salaries & Wages	2702000 00 *	83000 00 *	273000 00 *	300000 00 *	330000 00 *	363000 00 *	272052 00 *	635256 00 *	515544 00 *	142000 00	2913852.00
2	Chemical/Consumables	2284000 00	96 645 75	277263 23	301327 29	357437 33	276507 91	277875 00	376677 00	380274 00	179507 00	2523514 51
3	Travel	717000 00	18538 00	92929 10	92838 00	100404 00	90440 00	70609 00	176552 00	123978 00	21979.00	788267 10
4	Equipment	3260000 00	--	1619252 00	1429482 00	235399 00	--	–	–	–	–	3284133 00
5	Contingencies @ 10%	896000 00	19818 37	226244 43	212364 73	102324 03	72994 79	62053 60	118848 50	101980 00	34349 00	950977.45
		9859000.00	218002 12	2488688.76	2336012 02	1125564 36	802942 70	682589 60	1307333 50	1121776 00	377835.00	10460744 06

* As per sanction

Annexure 4

List of Equipments Purchased Under the Project

Sl No.	Name of Equipment
1	Lab Shaker
2	NSW - 191 Caltan Seed Germinator
3	NSW - 127 Water Bath Rectangular
4	NSW - 125 Thermostatic Water Bath
5	NSW - 143 Oven Universal
6	Gas Chromatograph Data Station with Accessories
7	Fridge 300 ltr.
8	Refrigerated Centrifuge
9	Chromatography Chamber
10	HPLC System with Accessories
11	Model 5000 Near Infrared Monochromator with Accessories
12	Remi Centrifuge R-23
13	Photoperiodic Simulator with Switching Units

Annexure 5

Technical programme:

- A large number of *B. juncea* and *B. napus* lines/varieties will be raised from open pollinated seeds. The self seeds of each line/variety will be harvested during rabi (1992-93).
- Selfed seeds of each line will be analysed for their erucic acid and glucosinolate contents (1993, Summer).
- Lines/varieties having low erucic acid and low glucosinolate of both the species will be grown with other high yielding adapted varieties and will be crossed to obtain F_1 seeds (1993-94).
- F_1 seeds will be analysed for oil quality and grown in off season nursery to collect selfed F_2 seeds (Summer, 1994).
- In addition, anther culture will be attempted to obtain double haploids. The individual double haploid plants will be analyzed for oil quality to select plants with low erucic and low glucosinolate. The selected plants will be evaluated in the field.
- Selfed F_2 seeds would be analysed for oil quality by half seed method at the time of sowing and seeds showing low erucic acid would be grown and selfed to get F_3 seeds (1994-95).
- F_3 seeds of individual selected F_2 plants will be analysed for oil quality (1995 Summer).
- Individual plant progenies of selected F_3 seeds will be grown and selfed. (1995-96).
- F_4 seeds will be collected and analysed for oil quality (Summer, 1996).
- F_4 plants will be grown on the basis of analysis to get superior lines having low erucic acid and glucosinolates (1996-97).
- F_5 seeds of selected superior lines or plants will be analysed for oil quality (Summer, 1997).